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CONTRIBUTIONS TO OUR KNOWLEDGE OF AMERICAN CARBONIFEROUS FLORAS

X. An Osmundaceous Stem from Iowa[1]

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Somewhat over a year ago we received from Dr. L. R. Wilson a collection of coal balls which had been obtained from the coal mine of the What Cheer Clay Products Co., What Cheer, Iowa. The petrifactions contain some especially well-preserved plant remains, among them undescribed Pteridosperm stems, lycopod leaves, and other fossils that will be dealt with in future contributions. The subject of the present account is a fragment of a small stem which we believe to be a very early member of the Osmundaceae. Although the central part of the specimen is well preserved the outer cortical tissues, as well as the great cloak of petiole bases, so characteristic of the family, are missing. While this renders impossible a comparison with a few fossil genera known only from outer cortical tissue and petiole bases (*Bathypteris rhomboidea* and *Anomorrhoea Fischeri*, in Kidston and Gwynne-Vaughan, 1909), it does appear to represent a significant link in our knowledge of the family, or the complex from which it originated. Moreover, a rather careful search through the collection has failed to reveal additional specimens, and since the mine has been abandoned our only course seems to be to record such evidence as is available.

Kidston and Gwynne-Vaughan presented in a series of four papers (1907-10) a monographic treatment of the petrified stem remains of plants referred to the Osmundaceae. These fossils are from widely scattered localities and range in time from the Miocene down to upper Permian horizons. The specimen described herewith is believed to be sufficiently distinct to be designated as a new genus, and if our concepts of its relationships to the fossils of Kidston and Gwynne-Vaughan be correct it extends the range of the family back into upper Pennsylvanian times. Use of the taxonomic category "family" may be questioned with justification even though it is a rather remarkable series of fossils, and a few

[1]Issued September 20, 1948.

comments on the inter-relationships of the various genera will be offered following the description of the specimen.

Protoösmundites Wilsonii gen. et sp. nov.

The single specimen consists of a well-preserved siphonostele and a portion of what apparently was the inner parenchymatous cortex, including numerous leaf traces.

The wood is cylindrical, 2 mm. in diameter, there being no evidence of cambial activity (fig. 1). Within this is a well-preserved core of parenchymatous pith. As may be noted in the transverse and longitudinal sections (figs. 1, 2, 6), the pith cells are essentially isodiametric and uniformly thin-walled, no evidence of tracheidal pitting being apparent. Since the preservation is good there seems to be no doubt that we are dealing with a clearly defined siphonostele and a purely parenchymatous pith. This has an important bearing on the position that the fossil occupies in the Osmundaceae, a point that will be considered later.

The xylem is very slightly crushed but was apparently perfectly cylindrical in life, and approximately .5 mm. in radial thickness. It is composed entirely of scalariform tracheids[1], there being no admixture of parenchyma cells. The protoxylem elements appear as numerous, slightly extruding groups around the outer periphery. The first-formed cells are very nearly exarch, but their exact position is obscured by the imperfect preservation in this region and by the abundance of leaf traces. However, the latter appear uniformly centrarch immediately after their departure (fig. 5), and since they depart in such rapid succession it is not possible to distinguish protoxylems of the stele from those of the traces.

The protoxylem cells are markedly smaller than those lying immediately within and could not have been more than 2 or 3 cells of being exarch in position. The xylem cells range from 12 μ in diameter for the protoxylem elements to more than ten times as large for the largest (innermost) metaxylem cells. Two of the latter (fig. 1) measured 180 x 120 μ and 220 x 100 μ.

The secondary thickening of the tracheidal walls (fig. 4) is uniformly scalariform with the exception of the small outermost cells, which may best be termed annular. The latter differ from the metaxylem cells in that the secondary thickenings consist of finer rings and apparently lack any border.

[1] In Part III of their series on the Osmundaceae, Kidston and Gwynne-Vaughan state that the xylary elements in both recent and fossil species of the group are actually vessels since perforations exist between pits of adjoining cells, and Gwynne-Vaughan considered this problem more generally in another paper (1908). We are inclined to doubt that the perfection of preservation in all fossils assigned to the family allows positive determination on this point. Even though the central membrane did disintegrate with maturation of the cells, to apply the term vessel would be misleading since in other respects these cells are more closely comparable with normal scalariform tracheids of other vascular cryptogams and gymnosperms than with the vessels of the angiosperms.

The leaf traces departed slowly, forming a very acute angle with the stele, as is clearly shown in radial longitudinal sections (fig. 6). This is also indicated in transverse sections, where, at any one point, a large number of traces may be noted (fig. 1). It is unavoidable that most of the traces are cut more or less obliquely, yet where nearly perfect transverse sections are available it is clear that the protoxylem occupies a central position (fig. 5). The secondary thickening of the leaf trace elements is the same as that of the outer stelar cells, consisting of very fine annular bands (fig. 3).

Aside from the leaf traces, the only extra-stelar tissue that is preserved is a portion of the inner parenchymatous cortex, the cells of which are uniformly thin-walled and of essentially the same shape as those of the pith although considerably smaller (figs. 1, 6). The decayed area between this tissue and the xylem was probably occupied by phloem, pericycle, and the innermost border of the cortical tissue.

Diagnosis of Protoösmundites: Stem with a small siphonostele of nearly exarch protoxylem tracheids, large scalariform metaxylem tracheids, and a parenchymatous pith; no secondary wood and the primary wood composed of tracheids only; leaf traces small and numerous as in *Osmunda* with a central protoxylem, the cylindrical form of the trace being retained for some distance through the cortex.

Locality: Coal mine of the What Cheer Clay Products Co., one-half mile west of What Cheer, Iowa.

Horizon: Des Moines Series, Pennsylvanian.

Discussion:

The affinities of this fossil appear to lie with the early representatives of the Osmundaceae. In order to clarify our views concerning this probable position it seems desirable to review very briefly certain of the fossils described by Kidston and Gwynne-Vaughan in their monograph on the Osmundaceae.

Six species of *Osmundites* are described from widely separated localities extending from the Jurassic into early Tertiary horizons. In the lower Pliocene (or upper Miocene) *Osmundites Schemnitzensis* from Hungary, and *O. Dowkeri* from the lower Eocene of Herne Bay, Isle of Wight, the xylem cylinder consists of separate strands and surrounds a parenchymatous pith. These relatively recent species are strikingly similar to the modern members of the Osmundaceae. In the upper Jurassic *O. Kolbei* from Cape Colony a comparable xylary structure is present but of special note is the presence of irregularly shaped tracheids in the central tissue, the latter being, in fact, a mixed pith. In the Jurassic *O. Dunlapi* from New Zealand the stele differs from all of the more recent species in having a continuous xylary ring. However, as the central tissue was not present, it is not known whether it was strictly parenchymatous or mixed.

Still earlier genera which are assigned to the family, *Zalesskya* and *Thamno-*

pteris, from the upper Permian of Russia, possessed a stele that was differentiated into two xylary zones. In *Zalesskya gracilis* it consists of an outer ring of elongate tracheids and an inner zone of shorter, nearly square-ended, pitted elements. *Z. diploxylon* is similar, although the contrast between the two xylary zones is more marked, the elements of the central xylem being of relatively greater diameter, shorter, and with transverse walls. In *Thamnopteris Schlechtendalii* the central xylem consists of reticulately pitted cells in contrast to the more regular porose cells of *Zalesskya gracilis*. It should be noted that in all three of these species the centermost portion of the stele had been lost through decay. However, Kidston and Gwynne-Vaughan note that: "As regards *Zalesskya diploxylon*, at any rate, we feel convinced that the central xylem occupied the whole of the center of the stele in the living plant. Further, we accept the deduction suggested by this conclusion, *that the vascular anatomy of the Osmundaceae must be derived from a protostele with a solid central homogeneous xylem mass.*" (II, p. 229).

This group of fossils, similar in the organization of their petiole structure, seems to present, through the evolution of a protostele to a specialized siphonostele, a clear-cut line of Osmundaceous ancestors going back to the upper Permian. Although *Protoösmundites Wilsonii* appears to lie closer to this alliance than any other series of ferns it is apparent that it does not fit perfectly into the sequence. If *Zalesskya* and *Thamnopteris* are typical of the family for the period one would expect, in this upper Pennsylvanian species, a somewhat less advanced parenchymatization of the pith. Furthermore, *Protoösmundites* differs from the previously described genera in that the scalariform thickenings of the xylary elements extend across the lateral wall rather than being separated into two or more series. It is not surprising, however, that in this earliest representative a somewhat simpler organization should prevail in this respect.

With regard to the leaf traces, it seems especially significant to note that *Osmundites skidegatensis* (Lower Cretaceous of British Columbia), considered to be the most advanced species, living or fossil, presents a leaf trace "very large, and it is already strongly curved, even while still in the parenchymatous inner cortex of the stem." (Kidston and Gwynne-Vaughan, 1907, p. 772). This is quite in contrast to the upper Permian genera *Zalesskya* and *Thamnopteris*, where the traces remain oval-shaped with a deeply immersed protoxylem for some distance through the inner cortex. Judging from a comparison of our specimen with Kidston and Gwynne-Vaughan's figures of these Permian genera the retention of the centrarch form of the trace is even more pronounced in the Iowa fossil. While further comments can be little more than speculative it appears that the petiole structure in *Protoösmundites* is distinctly primitive, pointing to a frond that was correspondingly less specialized in its general morphology. Until further evidence may confirm or refute it we are inclined to look upon this fossil as either an early representative of the Osmundaceae proper or a member of an associated line leading up from the Coenopterid complex.

Acknowledgement:

Grateful acknowledgment is made to Prof. L. R. Wilson for his donation of the coal balls on which this study was based.

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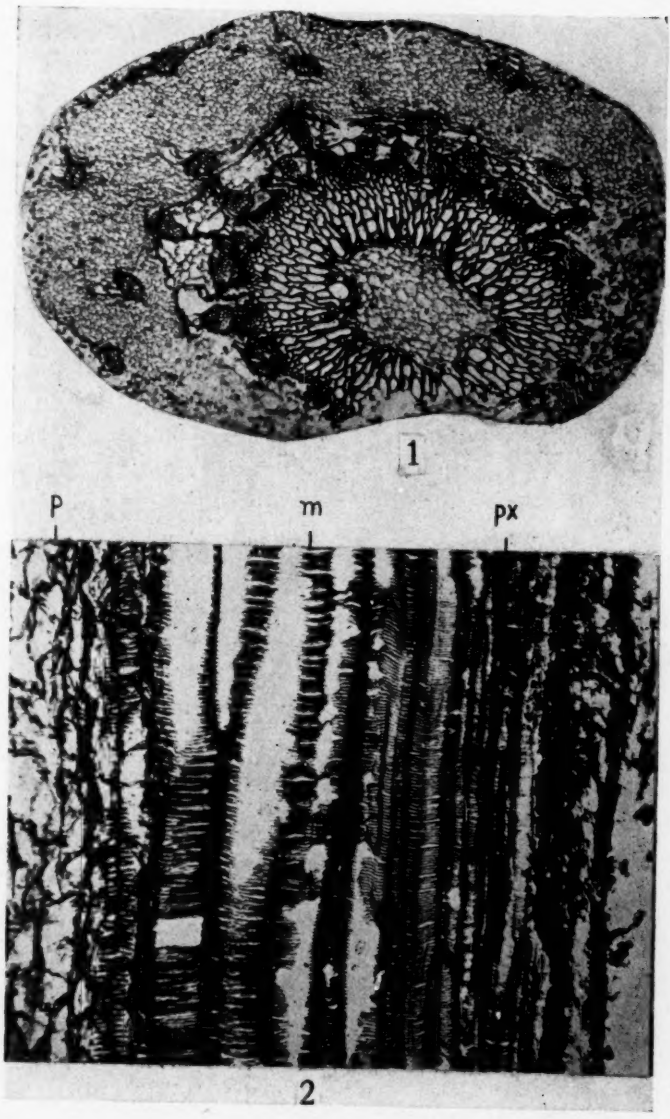
EXPLANATION OF PLATE

PLATE 9

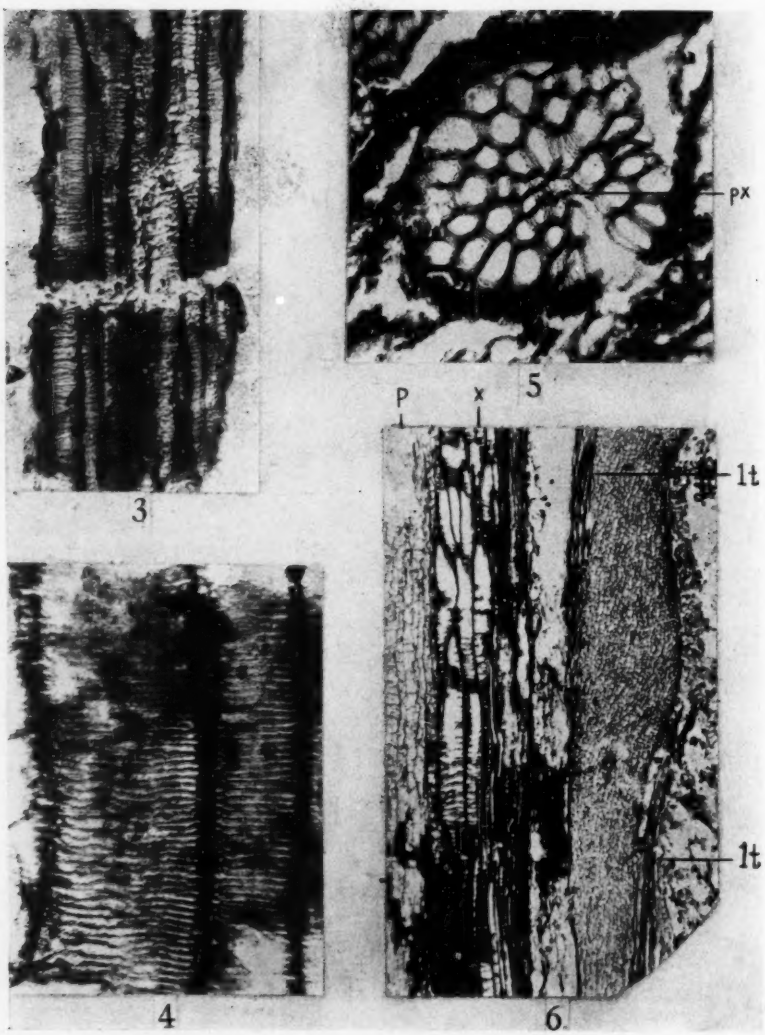
Protoösmundites Wilsonii

Fig. 1. A transverse section of the specimen showing central pith, wood, leaf traces and parenchymatous cortex. Slide 1521, × 21.

Fig. 2. A radial longitudinal section showing the scalariform nature of the xylem elements: *p*, pith; *m*, metaxylem; *px*, protoxylem. Slide 1518, × 100.



ANDREWS & BAXTER—*PROTOOSMUNDITES WILSONII*



ANDREWS & BAXTER—*PROTOOSMUNDITES WILSONII*

Explanation of Plate

PLATE 10

Protoösmundites Wilsonii

Fig. 3. A leaf trace in longitudinal section showing the fine annular thickenings. Slide 1518, × 300.

Fig. 4. Radial view of two metaxylem tracheids. Slide 1515, × 210.

Fig. 5. A leaf trace shortly after its departure from the stele: *px*, protoxylem. Slide 1520, × 230.

Fig. 6. A radial longitudinal section through the stem: *p*, pith; *x*, xylem; *lt*, leaf trace. Slide 1514, × 11.

A CROSSOTHECA FROM NORTHERN ILLINOIS

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During a collecting trip to the famous nodule-producing strip mines south of Chicago, in April, 1947, a specimen of *Crossotheca* was obtained which displays both fertile and sterile portions of a frond in organic connection. From a comparison with the American and British fossils assigned to this genus our specimen appears to represent a new species.

A good deal of interest has been shown in *Crossotheca* and supposedly related fossils since it is felt that they are microsporangiate fructifications of certain Pteridosperms. The general problem of the affinities of these fructifications has been considered at length by a number of previous workers and need not be repeated in detail here. About a dozen species of *Crossotheca* have been described, and while certain of these are imperfectly known with reference to the structure of the synangia, the morphology of the sterile foliage, and the general organization of the frond, it is evident that it was a large and varied genus. Judging from the work of Lesquereux, Kidston, Crookall, and others it seems clearly established that in some species the sterile portion of the fronds was of the *Pecopteris* type and in others of the *Sphenopteris* type.

It is not possible to make satisfactory comparisons with all of the previously described species since in some instances spore measurements are not given or the sterile pinnules have not been found attached.

Crossotheca McLuckiei sp. nov.

The single specimen on which this description is based consists of the terminal portion of a fertile penultimate pinna with portions of three sterile pinnae at its base. It is not possible to say what fraction of the entire frond is represented but it is significant that the sterile and fertile portions are in organic connection.

The entire specimen is 6.5 cm. long (fig. 1), including the sterile portion at the base. It is terminated by a fertile pinnule, below which lie five or six lateral single pinnules, and below these are pinnae consisting of from three to five fertile pinnules each. The base of the specimen is terminated by three sterile ultimate pinnae, two of which are shown in fig. 2.

The fertile pinnules are about 3 mm. long and 1 mm. wide and apparently consist of a much-reduced "lamina" bearing up to 20 sporangia in the usual marginal fashion for *Crossotheca*. The sporangia are not well preserved, but when shown in side view appear to have not exceeded 1.5 mm. in length. The spores are mostly uniform in size, being spherical, 70 μ in diameter, with a distinct trilete commissure and a very faintly warted exine (fig. 5). A few spores have been observed that are appreciably smaller (fig. 6) than the others, these measuring 45μ.

[1] Issued September 20, 1948.

Origin: Collected from an old "spoil" near the entrance of the miner's Recreation Area, north of Coal City; base of the Carbondale formation, middle Pennsylvanian.

Type specimen: No. 5005, preserved in the paleobotanical collections of the Henry Shaw School of Botany.

Discussion:

In 1902 Sellards described two species of *Crossotheca* from Mazon Creek, *C. trisecta* and *C. sagittata* (*Staphylopteris sagittatus* Lesquereux). The fertile pinnules, as a whole, as well as the sporangia, of *C. sagittata* are much larger than those of *C. McLuckiei,* and the fertile foliage appears to be more distinctly pecopterid in form. *C. trisecta* differs in that its spores are almost exactly half the size of those from our specimen, and the sterile foliage, according to Sellards, is similar to that of *C. sagittata.* There seems to be no question that the specimen described here is distinct from these two.

Of the Crossothecas considered by Kidston in his monographic review of the genus (1923) *C. communis* (Lesquereux) Kidston seems to be the most closely comparable to our specimen. According to Kidston this species is rare in Britain and was originally based on sterile *Sphenopteris* foliage from American material. The sterile pinnae of *C. communis* (see Kidston, pl. 89, fig. 9b) resemble our specimen but unfortunately measurements of the sporangia and spores are not given for *C. communis.*

Whether all of the fertile specimens that have been described as *Crossotheca* are naturally referable to a single genus may be questioned. Being compression species there are certain structural details about which we would like further knowledge. However, it seems likely that all are closely comparable in the general organization of the fertile pinnule. Although there is appreciable size difference in the spores, their spherical shape and the "minute warty thickenings" that characterize the wall are remarkably uniform in most species. On the other hand, the range in form of the sterile pinnules is equally striking. In *C. Schartzlarensis* the pinnules are very finely divided similar to those of *Rhodea;* in *C. communis, C. McLuckiei,* and *C. Hoeninghausi* they are of the *Sphenopteris* type; in *C. pinnatifida* they are closer to *Neuropteris;* and in *C. Boulayi* closer to *Pecopteris.*

The specimen is named for Mr. John L. McLuckie in recognition of his continued interest in the fossils of the Mazon Creek region and for his generous services as guide on numerous collecting trips.

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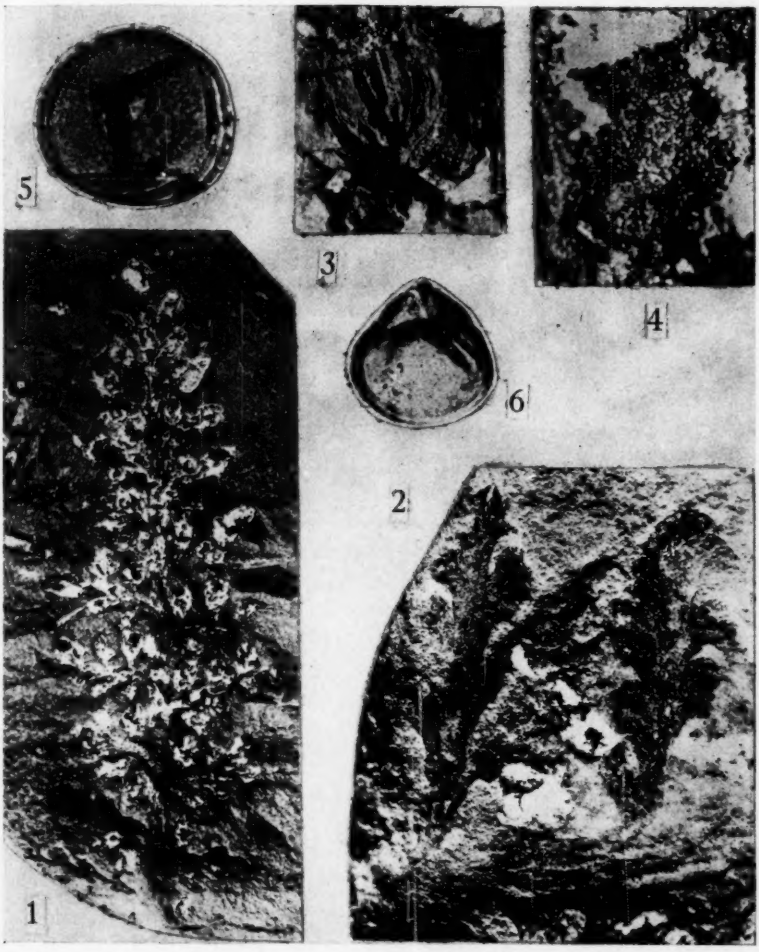

EXPLANATION OF PLATE 11

Crossotheca McLuckiei

Fig. 1. The entire specimen enlarged × 1.5.
Fig. 2. Two sterile ultimate pinnae shown at the lower left of fig. 1, × 4.5.
Fig. 3. A fertile pinnule from which most of the spores have been shed.
Fig. 4. Portion of a fertile pinnule showing crushed sporangia in side view.
Fig. 5. A representative spore, × 400.
Fig. 6. A smaller spore (see text), × 400.

A NOTE ON *FOMES IDAHOENSIS* BROWN

HENRY N. ANDREWS

About a year ago two specimens of the late Tertiary polypore, *Fomes idahoensis* Brown, were described (Andrews and Lenz, 1947) which had been collected the previous summer south of Bruneau, Idaho. In the summer of 1947 the same locality was re-visited by Mr. S. H. Osgood, of Rupert, Idaho. He obtained a number of additional specimens all of which are referable to the same species, although they represent much larger sporophores than have been reported formerly and indicate quite clearly that they were perennial plants.

Of these newly acquired specimens, one (No. 5002) is a fragment of a sporophore in the first or possibly second year of growth, the maximum length of the pores being about 28 mm. long. This specimen measures approximately 14 by 6.5 cm., being somewhat larger than previously described ones although it is not a complete bracket.

Of particular interest are specimens No. 5003 and No. 5004, which are pore fragments only. While these retain none of the context and offer no clues as to the size of the bracket as a whole the pores in both attain a length of 70 mm. Judging from the pore size these specimens, fragmentary though they are, compare closely with the previously described specimens (Andrews and Lenz, 1947), as well as the type material (Brown, 1940), from the same locality. They seem to offer conclusive proof that the original designation of these fossil polypores to the genus *Fomes* (Brown, 1940) was correct. There is a suggestion of "rings" in the pores (No. 5003), indicating four or five years of growth, although these cannot be clearly distinguished.

The author gratefully acknowledges Mr. Osgood's generosity in donating these interesting and rather rare fossil fungi to the collections of the Henry Shaw School of Botany.

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A STUDY OF THE VEGETATIVE ANATOMY OF THE GENUS SPHENOPHYLLUM FROM AMERICAN COAL BALLS

ROBERT W. BAXTER

Introduction

Although the genus *Sphenophyllum* has been described in some of the earliest paleobotanical works and Seward (1898) lists it as one of the best-known fossil plants, the rich source of petrified specimens in the American coal balls has been very largely ignored. The works of Renault and Williamson (1878) did much to make known the inner structure of the stems, roots and leaves of the English and European forms, although gaps were left concerning our knowledge of branching, the attachment of roots, and other anatomical points. It was with the hope, therefore, of contributing to our knowledge of the internal structure of the plants, as well as the desire to make better known the American fossils and to correlate them in so far as possible with the English and French species, that this study was undertaken.

The material on which this study was based was obtained from two sources as follows: The Pyramid mine, two miles south of Pinckneyville, Perry County, Illinois, this horizon being at the base of the McLeansboro series (coal No. 6) and of upper-middle Pennsylvanian age; and The What Cheer Clay Products Co. coal mine, one-half mile west of What Cheer, Iowa. This horizon lies in the Des Moines series of the Pennsylvanian.

Review of Literature

Sphenophyllum, in the form of compressions, has been noted and described by some of the earliest investigators. Solms-Laubach (1891) states, "The genus, owing to its striking appearance, has been repeatedly figured by the old authors." In Scheuchzer's *Herbarium Diluvianum* (1723) there are drawings of stem fragments bearing whorls of small wedge-shaped leaves which were undoubtedly made from specimens of *Sphenophyllum*.

Renault (1878) was the first to link together definitely the petrifactions showing internal anatomy with the well-known leaf and stem compressions. He described three species from petrified material, only one of which, S. *quadrifidum*, has been retained by recent authors. It is supposedly characterized by double groups of protoxylem at each angle of the primary triangle, giving a hexarch structure. However, in examining Renault's original plates and descriptions we have found little evidence of clearly defined hexarch anatomy. His other two species based on petrifactions were S. *stephenense*, which is figured as having two forked and two single leaf traces from each protoxylem angle, and S. *erosum*, which his drawing purports to show with 18 leaf traces radiating out from the stele in all directions. Whatever the 18 "rays" were in the later drawing, we feel certain

that they were not leaf traces though it is possible that they could have been vascular strands to adventitious roots.

Williamson (1874) described two petrified stems with triarch protosteles as species of *Asterophyllites*. In 1895, recognizing their true nature, he redescribed them in detail and with many excellent illustrations, as *Sphenophyllum plurifoliatum* and *Sphenophyllum insigne*. *S. plurifoliatum* did not differ in any important anatomical features from the specimens previously figured by Renault, but Williamson's detailed descriptions clarified the characteristic structure of the genus. *S. insigne* differed in coming from a much lower horizon in the Lowest Carboniferous, supposedly in having fairly consistent continuous parenchyma rays in the interfascicular[1] wood, and in the presence of protoxylem lacunae.

Koopmans (1928) has described two new species, *S. minus* and *S. perforatum*, from the Finefrau horizon of the Netherlands. The former differs from *S. plurifoliatum* in having a smaller protoxylem strand (0.4 mm.), a less concave metaxylem, a narrower fan of fascicular xylem, and smaller interfascicular xylem cells adjoining the metaxylem. *S. perforatum* differs in possessing protoxylem lacunae, smaller metaxylem cells, and more prominent arms to the primary wood triangle. We shall have more to say concerning these species later.

Leclercq (1925) has described *S. Gilkineti* from the upper Carboniferous of Belgium on the basis of two different zones of secondary wood. This species will be treated more fully below when variations of the *Sphenophyllum* stem are described.

With the exception of the six species just mentioned, all of the other *Sphenophyllum* species have been described on the basis of leaf differences observable in compressions. Potonié (1910) lists eight species, while Lesquereux (1880), in his work on the coal flora of Pennsylvania, describes nine variations in leaf form. Five of these he assigns to European species, intimating a close relationship for the plant in the two geographical areas, a fact which finds support in petrified material. Indeed Walton (1940) illustrates (his figure 42) a *S. plurifoliatum* stem type from England which is so similar to the Illinois material that it could have been made from one of our own peels.

Prior to this study there has been almost no attention paid to petrifactions of *Sphenophyllum* in this country. A few investigators have mentioned finding stems of the *S. plurifoliatum* type in American coal balls but no descriptions have been given. Darrah (1939), in dealing with the flora of Iowa coal balls, listed *Sphenophyllum* stems and strobili (of the *S. Dawsoni* type) but did not include any illustrations or descriptions. This neglect is the more surprising since the Iowa and Illinois coal balls contain abundant and excellent specimens. It is a rare coal ball that does not produce at least one or two stems, while some are found with as

[1] The terms fascicular and interfascicular are used here and throughout the text in the sense originated by Williamson and Scott (1895): fascicular, in all cases, referring to that secondary wood formed opposite the protoxylem groups; and interfascicular indicating the secondary tissue formed opposite the sides of the triangular protostele and between the protoxylem groups.

many as nine or ten stems in an area 3–4 inches in diameter. Consequently we have had the advantage of being able to observe hundreds of specimens in varying stages of growth.

General Description

The external appearance, as evidenced in compressions, is that of a slender stem 1 cm. or less in diameter, with cortical furrows and whorls of leaves which do not alternate from node to node as in *Calamites*. (The most ancient of the *Calamites, Archaeocalamites,* from the Lower Carboniferous did, however, have the same superimposed structures.) The nodes themselves are commonly somewhat swollen while the leaves vary considerably in size and form, a large number of species having been described on the basis of foliar differences. The type on which the genus was founded has wedge-shaped leaves with an entire or very slightly toothed margin. In some species the foliage is deeply dissected, while in others the plants are heterophyllous, bearing both entire and deeply lobed leaves on the same stem. The anatomy of the stem has constituted one of the primary reasons for the isolation of the genus.

Primary Tissues

Transverse sections of the stems show the primary wood to be a solid mass of tracheids, triangular in outline and with the protoxylem occupying the apices of the triangle. The protoxylem tracheids are small (20 μ in diameter), with ring or spiral thickening. The transition to metaxylem is quite abrupt, with a distinct increase in cell size and a characteristic reticulate bordered pitting on both the radial and tangential walls.

Generally most of the plants observed appear to comply with the descriptions of *Sphenophyllum plurifoliatum* Williamson. However, we shall point out several variations from the basic *plurifoliatum* type which seem to indicate that stem anatomy, at least in the internodal region, may constitute a doubtful basis for specific differences.

On the whole, our Illinois specimens are smaller than those described from England and Europe. The largest stem observed was not over 4 mm. in diameter, while Williamson (1895) has indicated that many of the English specimens reached 1 cm. It is of some interest also to note that while Bower (1930) observed primary wood averaging 1 mm. in width (measured from a protoxylem angle across to the opposite fascicular wood) the largest we have been able to secure measures .5 mm., the average size being .4 mm. The Iowa stems (figs, 3, 7, 8, 10 and 11) show the same small primary wood, though some of them (figs. 7, 11) exhibit considerably more secondary wood with larger total diameter than was found in the Illinois specimens.

Immediately adjoining the primary wood there is often a layer, usually one cell thick but sometimes two, of xylem parenchyma which surrounds the entire triangle with the exception of the protoxylem angles (fig. 17).

Figure 1 illustrates a stem which presents the features of *S. plurifoliatum* although showing clearly defined protoxylary canals which hitherto have been observed only in the more ancient *S. insigne*.

The lacunae appear to be the result of a disintegration of the protoxylem tissues, as very often an accompanying disruption is to be noted in the adjoining fascicular wood. From the constancy of their occurrence there is little doubt but that they represented a character of the living plant. Figure 1, showing one of the few stems of this type retaining its epidermis (most of the specimens having only a thick periderm), exhibits deeper and more numerous furrows (in this case eight) than are usual in *S. plurifoliatum*, which commonly has just three deep grooves opposite the sides of the primary triangle. However, none of these specimens just described was found with leaves or branches, and because our studies on the external form of the stem at the node and internode in a definite *S. plurifoliatum* stem showed that there could be very wide differences in size and form it seems wiser, at least for the present, to list this group as one of the variations of the basic *plurifoliatum* type than to attempt to assign it specific value.

Secondary Tissues

The secondary wood is radially arranged around the triangular primary tissues in a geometrical pattern that results in the rows of cells opposite the protoxylems being much smaller than those opposite the metaxylem. Consequently the fascicular wood forms a radiating fan of narrow rows of small cells which gradually increase as their angle of divergence increases until in the outer margin of large stems the two zones are almost identical (fig. 11). According to Williamson (1895) and Scott (1920), this fascicular region has continuous parenchyma rays of a different type from those of the interfascicular wood. However, we have been unable to observe any evidence of this in our material, while indeed some stems (fig. 11) very obviously show identical ray structure to the interfascicular zone.

The tracheids of the interfascicular xylem are in transverse section normally large, rectangular, and with truncated angles, the spaces between them being occupied by groups of small, vertically aligned parenchymatous cells. In radial section these vertical parenchyma cells are connected by many small horizontal ones extending across individual tracheids, forming an effective ray system (fig. 8).

Figures 5 and 6 show an interesting stem in which an injury evidently caused the growth of "fascicular type" wood for over two-thirds of the circumference. The injury appears to have occurred when two rows of secondary wood had been formed and to have caused the cambium to start producing a series of smaller cells. On the side of the stem farthest from the wounding the secondary wood was unaffected. The abnormality is evidently quite similar to *Sphenophyllum Gilkineti* Leclercq, concerning which we shall say more later.

Another secondary wood variant is illustrated in fig. 2. Here an apparent sclerotic growth is seen replacing the usual corner groups of parenchyma. The

Sphenophyllum nature, however, is still obvious in the triarch structure of the primary wood, the deep-seated periderm, and the sclerotic furrowed outer cortex.

The pitting of the secondary wood is similar to that described for the metaxylem except that there are fewer pits in the tangential walls. The pitting presents a good character for recognizing the genus in longitudinal sections, the pits being ovoid to circular and arranged so thickly on the radial walls that they form a reticulate pattern. All previous investigators have described these pits as bordered, but it seems likely that the border must have been quite fragile, since the pits normally appear simply as perforations in the cell wall (fig. 8). Next to the pitting the most distinctive character of the wood is the length of the xylem cells. Although longitudinal sections over 1 cm. long were obtained, it was still impossible to find any trace of end walls, so the question of the true nature of these cells (tracheids or vessels) must remain an open one.

The parenchyma tissues outside the wood are seldom well preserved, being replaced at an early stage by a deep-seated periderm which appears to have arisen first in the pericycle or endodermis and then in successively deeper series within the phloem. Figure 12 illustrates a cross-section showing two layers of periderm, the inner abutting almost directly on the secondary wood so that only a few fragments of phloem and cambium remain. In longitudinal view the periderm can be seen to consist of regular rows of short parenchymatous cells, darker in color than the other stem tissues and appearing to retain considerable cell contents.

The outer cortex in mature stems is often replaced by the thick periderm growths just described. When present it offers a distinctive character in its strongly sclerotic appearance and furrowed outline.

Branching

To our knowledge, branching in the petrified material of *Sphenophyllum* has not been reported up to this time. Williamson (1874) illustrated a specimen showing the base of a lateral appendage, although it seems most likely that it represented a root departure. The vascular tissue followed a horizontal course outward from the stele which, as will be pointed out, appears to be characteristic of the leaves and roots but not of true branches. It was with particular interest, therefore, that we found in the specimens represented in the Illinois and Iowa coal balls three stems exhibiting clearly defined branching, each quite different and distinct.

The first stem to be described is characterized in the internode by the roughly hexagonal shape of the outer cortex and an oval periderm surrounding the triangular woody tissue (fig. 15). As the node is approached both the outer cortex and the inner parenchyma layers immediately surrounding the xylem become almost spherical (fig. 16). A short distance above this the general form molds itself to that of the woody cylinder and becomes triangular throughout (fig. 18). At this stage the first evidence of branching is seen in the out-thrusting of segments of pericycle and phloem tissue through the surrounding periderm and cortex

at points opposite the protoxylem angles. There also appear at this time swellings in the periderm, approximately along side of each of the three protoxylem groups, two of them on each side of the triangle. Next, the over-all shape of the stem becomes more definitely triangular and horizontal vascular strands appear extending out from the protoxylem into the three corners and also into the six swellings on the sides (fig. 19). In the next peel (fig. 20) we see that the six side vascular strands lead into the bases of leaves while the fascicular tissues at the corners have extended through the outer cortex at a sufficient angle from the horizontal to exhibit most of their area in transverse view. These large corner vascular bundles, surrounded by a thin layer of phloem and pericycle and what appears to be tissue from the periderm, are soon pushed out beyond the outer cortex of the main stem. At this stage adventitious roots are produced abundantly and appear passing out through the cortex of these vascular bundles in all directions (figs. 20 and 22). The following stage finds the three branches separated from the main stem by their own sclerotic outer cortex. The numerous adventitious roots are still evident while the stem is observed to have regained its original hexagonal form (fig. 21). Our last illustration of this series shows the central portion of one of the branches, some distance from the main stem, in which the characteristic triarch stele structure is beginning to appear (fig. 23).

In the second specimen the stem produced just one branch instead of three, and there is no evidence of a node or leaves in the vicinity of the branching. The offshoot is first observed as a large mass of fascicular wood coming off from a protoxylem corner of the primary triangle. Its angle of departure from the stem is quite acute so that it maintains an approximate vertical position throughout its passage. The main stem, instead of the triangular shape characteristic of our other specimen, is a flattened oval with the elongation in the direction of the branching (fig. 26). The preservation of the branch was so very poor shortly after it became independent that it was not possible to follow it for a sufficient distance to show a node; however, fig. 27 illustrates its appearance at the point of separation from the stem. The typical sclerotic furrowed outer cortex is evident, and while the primary wood does not as yet exhibit the usual triangular form of our previous specimen (in which the preservation was considerably better) this character did not develop until some distance from the main stem. Probably the most curious feature of this specimen is the production of two curved appendages from the other two corners of the primary triangle at the same vertical position as the start of the branch trace (fig. 25). The vascular tissue in these appendages is horizontal (90° angle to the main stem) and the structures themselves are apparent for some distance in a horizontal position. They are quite small and with a thick cortex, at least at the base where they depart from the stem, so they obviously could not be leaves. We are, therefore, inclined to regard them as modified roots which functioned as specialized appendages enabling the vine-like *Sphenophyllum* to cling or prop itself upon supporting surfaces.

The third form of branching observed is illustrated in figs. 7 and 10 and appears to have consisted of an unequal dichotomy with the branch being considerably larger than the main axis. That the larger structure constituted the branch was proved by longitudinal sections showing the angle of departure. This feature would seem to offer additional evidence of the vine-like nature of *Sphenophyllum*.

Leaves

The leaves were borne in whorls at the nodes and were fused for a short distance from the base (fig. 29). They were in multiples of three, and six appears to be the average number of the Illinois and Iowa specimens. In the six-leaved specimens vascular tissue was supplied by means of V-shaped traces given off from each angle of the primary triangle, one trace going into each leaf and dividing dichotomously in the outer cortex or the leaf base. Figure 3 shows an unusually perfect nodal section with all six leaf traces traversing the cortex into the leaf bases. In the destruction of the inner tissues the connection of the traces to the protoxylem angles has been lost; however, fig. 32, a nodal section of a smaller stem, comes fairly close to showing the attachment.

The internal anatomy of the leaf appears to have been relatively simple. The outstanding feature in the petrified material is the conspicuous ring of fiber strands enclosing the 7–8 tracheids which form the vein (fig. 24). The mesophyll seems to have been undifferentiated and seldom of more than one or two cells in thickness. The lower and upper epidermal layers were one cell thick and with no apparent cuticle. No stomata were observed though they have been reported by Renault (1878).

Roots

The roots were first identified and investigated by Renault (1878). The identification was made on the basis of relatively large roots which possessed the same distinctive secondary wood as the stems. Since then they have been noted and figured by various investigators but never organically connected with the stems.

Early in the present work it was noticed that surrounding many of the *Sphenophyllum* stems were small roots averaging .3 mm. to .4 mm. in diameter. These consisted of 2–7 small tracheids, a well-defined endodermis, 2–3 layers of large parenchymatous cortex cells (average diameter 50 μ), surrounded by a single-layered epidermis of conspicuously large cells (diameter 40 μ), with abundant root hairs (figs. 9 and 30). From two coal balls we were able to secure peels which contained, along with the *Sphenophyllum* stems, almost homogenous masses of these roots in which all sizes were represented from the smallest, described above, to mature roots such as illustrated in fig. 28. These showed a nearly continuous series of developmental stages, indicating conclusively that these small previously undescribed roots represent the initial stage of the already known mature specimens. Secondary growth appears to have been initiated very early and to have

produced rapidly the characteristic square tracheids with the groups of parenchyma cells at their angles. Since the cambial growth was from a small rounded primary strand the radiating rows of secondary wood are uniform and are not differentiated into fascicular and interfascicular zones as in the stem where the growth was from a triangular base. A phellogen also became active in the endodermis at an early stage and by the time that 4–5 layers of secondary wood had been produced the periderm had usually grown to the degree that the cortex and epidermis were lost. Thus these latter tissues are observable only in the youngest roots (fig. 30).

In none of our specimens do we find much support for the belief that these roots were diarch or even monarch in the strict sense. Figure 30 illustrates a small root in which there are approximately six tracheids composing the primary wood. There is no apparent growth of metaxylem, while there does seem to be, even at this early stage, the initiation of secondary growth as evidenced by the large tracheids to be seen on both sides of the primary tissue.

In order to establish beyond any doubt the *Sphenophyllum* origin of these roots particular attention was paid to finding some in actual connection with the stems. In making a longitudinal series of peels through a well-preserved specimen, the structure illustrated in fig. 35 was discovered. Here we have a root identical to the small ones described above, within the middle cortex of a *Sphenophyllum* stem. The conspicuous epidermis, large cortical cells, and dark endodermis are clearly recognizable. The evidence seems to be quite definite that the adventitious growth of these roots could and did occur on any part of the stem but that it was ordinarily most abundant in the vicinity of the nodes. As previously mentioned in the description of branching, many of these small roots were observed being given off at the base of the newly formed branches (figs. 21 and 22).

In addition to the above instances, figs. 31 and 33 show a longitudinal section of a *Sphenophyllum* stem with what we believe to be extremely large roots being given off opposite each other and immediately dividing into groups of small roots. Figure 31 shows the vascular strand passing horizontally from the stem into the appendage. It is felt that this is an important diagnostic character since, as indicated earlier, the vascular bundles passing to the branches always leave the stele at an acute angle. Therefore only the leaves and the roots exhibit this horizontal passage of the vascular tissue, and in this case we are clearly not dealing with a leaf.

The pitting of the tracheids of both small and large roots was observed in longitudinal radial sections. The reticulate bordered pitting, so common on the radial walls of the stems, was clearly evident in both.

Discussion

It is apparent that although *Sphenophyllum* has been described as "one of the best-known fossil plants" (Williamson and Scott, 1895), and has been illustrated by numerous authors since Renault's time, there still remain phases of its general organization that need clarification.

Our three stems, while presenting identical anatomy in the internode region, show quite distinct differences at the point where the branch or branches are given off. In one we have three offshoots at a conspicuous node, while in another just a single branch is produced with root-like appendages springing from the other two protoxylem angles and with no sign whatever of any node or leaves, and in the third there is an unequal dichotomy with the branch being the larger. In all three the branch vascular tissue originates from the main stele at an acute angle instead of a right angle, as is characteristic of the leaves and roots, and the first two specimens described show typical sclerotic cortex; while the specimen in which we were able to follow the branch for some distance presented a clearly outlined triarch protostele. Therefore we have either the anomaly of three distinct branching patterns on one plant or significant taxonomic characters which are not correlated with any other observed differences in stem anatomy.

It would seem that the anatomy of the internode (which makes up 99 per cent of the sections usually obtained) is an unreliable key to specific segregation, in that it fails to emphasize sufficiently such differences as may exist in other parts of the plant, or may, on the other hand, present misleading supposedly specific differences. Examples of this latter point are illustrated in figs. 2 and 5 where the variations are, we feel, due to some local outside influence.

Further difficulty in attempting to define additional species on stem anatomy is well shown in the specimen illustrated in fig. 15. Here the internodal structure is identical in every way to S. *plurifoliatum* but at the node is shown clearly to have only six leaves, while both Scott (1920) and Williamson and Scott (1895) state repeatedly that their specimens had many leaves, probably around 18. Lacking nodal sections they could not make an exact count but evidently they had considerable evidence from leaf parts preserved with the stem. Possibly they were working with a different species than we have illustrated, but one of strikingly similar wood anatomy.

All points considered, it seems more feasible, at least for the present, to allow the species S. *plurifolium* as described by Williamson and Scott to cover nomenclature needs of these Illinois and Iowa petrified stems. We do feel that a short description of the structural variations is worth giving here. Then, if additional research reveals correlating characters in other plant organs they could be given specific importance.

Type 1.—Characterized by constant presence of protoxylem lacunae. Protostele averages .4 mm. from angle to opposite side of triangle. Large metaxylem cells with occasional xylem parenchyma on margin. Outer cortex in internode region more or less circular in outline with 8–10 deep furrows (fig. 1). This type resembles S. *perforatum* and S. *insigne* in its possession of protoxylem lacunae, but in all other respects it is identical to S. *plurifoliatum*. The characters separating the aforesaid species from S. *plurifoliatum* are, in our opinion, doubtful.

In examination of Williamson's figures and of *Sphenophyllum insigne* peels from the Calciferous Sandstone horizon we have been unable to observe the continuous medullary rays in the interfascicular wood. This feature, along with the protoxylem lacunae, supposedly distinguished the species, while the slight differences in size and form of the primary wood listed by Koopmans for S. *perforatum*, in our opinion, are too variable to be reliable.

Type 2.—No protoxylem lacunae. Definite xylem parenchyma between metaxylem and interfascicular wood. Large metaxylem cells, equal in size or larger than secondary wood. Protostele size averages same as above. External form of outer cortex at the internode is hexagonal with a deep furrow opposite each side of triangular protostele. S. *minus* Koopmans would fall in this group. The differences in the concavity of the metaxylem, shape and size of the fascicular and interfascicular wood are not, we feel, constant enough in any of the forms to be reliable characters. The pure size difference of the protoxylem that has been observed in the Netherlands and Illinois and Iowa forms is not in itself sufficient reason for separating them from S. *plurifoliatum*.

Type 3.—Abnormal growths resulting from injury to stem (figs. 5 and 6) and sclerotic growths replacing usual corner parenchyma (fig. 2). Not really types at all but specimens which may show up with some frequency and which should be recognized for what they are. S. *Gilkineti* is, we feel, identical to the type showing abnormal growth resulting from wounding. Miss Leclercq's type specimen, as she stated, also had obviously been injured in growth and while she recognized the possibility of its being an abnormal growth her reasons for assigning specific value were as follows:

> Nous avons longtemps hésité dans l'interprétation des nos échantillons des figures 2 et 3. La présence de deux bois secondaires différents dans la tige complète de la figure 2 pouvait elle s'expliquer uniquement par l'excitation des tissus végétaux due aux buessures, ou représentait-elle la structure normale d'un nouveau type de *Sphenophyllum?* [p. 33].
>
> La découverte, dans le travail de Williamson, d'une coupe transversale très bien conserveé, d'un *Sphenophyllum* identique à celui de notre figure 2, a levé le doute quant à l'interprétation; nous sommes bien en présence d'une espèce nouvelle. Il est en effet invraisemblable de supposer que le *Sphenophyllum* de Williamson ait pu etre blessé lui aussi de telle manière qu'il reproduise une structure identique à celle de notre S. *Gilkineti.* [p. 33].

With our discovery of a stem showing the two zones of secondary wood accompanied by wounding it no longer seems "improbable" to suppose that Williamson's figure was also of an injured stem.

There is still much to be learned from more extensive studies of the petrifactions of *Sphenophyllum*. It is undoubtedly a larger group and more diversified than has been so far suspected. While the anatomy of the internode seems unreliable as a basis for specific distinctions (at least from the present material) the discoveries of additional nodal sections may be expected to show wide variations in leaf and branch form of possible specific value

The most important implications, we believe, to come out of the present study are additional facts for the relationship of *Sphenophyllum* to Equisitales. As Jeffrey (1899) points out "protostely and siphonostely may occur in different

genera of the same family, and even different species of the same genus." So with the observance in *Sphenophyllum* of fairly common protoxylem lacunae and origin of the branches *between* the leaves the only major distinction separating the groups is the peculiar parenchyma ray system of *Sphenophyllum*, and in this we have seen how occasional horizontal conjunction of the radiate parenchyma may produce structures similar to normal rays.

Therefore, on the basis of vegetative anatomy, we are inclined to agree with Jeffrey that there is no valid reason from excluding *Sphenophyllum* from the Equisitales and that it should be regarded as an offshoot from the group ancestor in which the primitive protostele and superimposed internode and leaf whorls have been retained.

Acknowledgment

Thanks are due Dr. Henry N. Andrews for his criticism and advice during the progress of the present work. We are also grateful for the continued goodwill of the Binkley Coal Company and their excellent cooperation in our collecting work.

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Explanation of Plate

PLATE 12

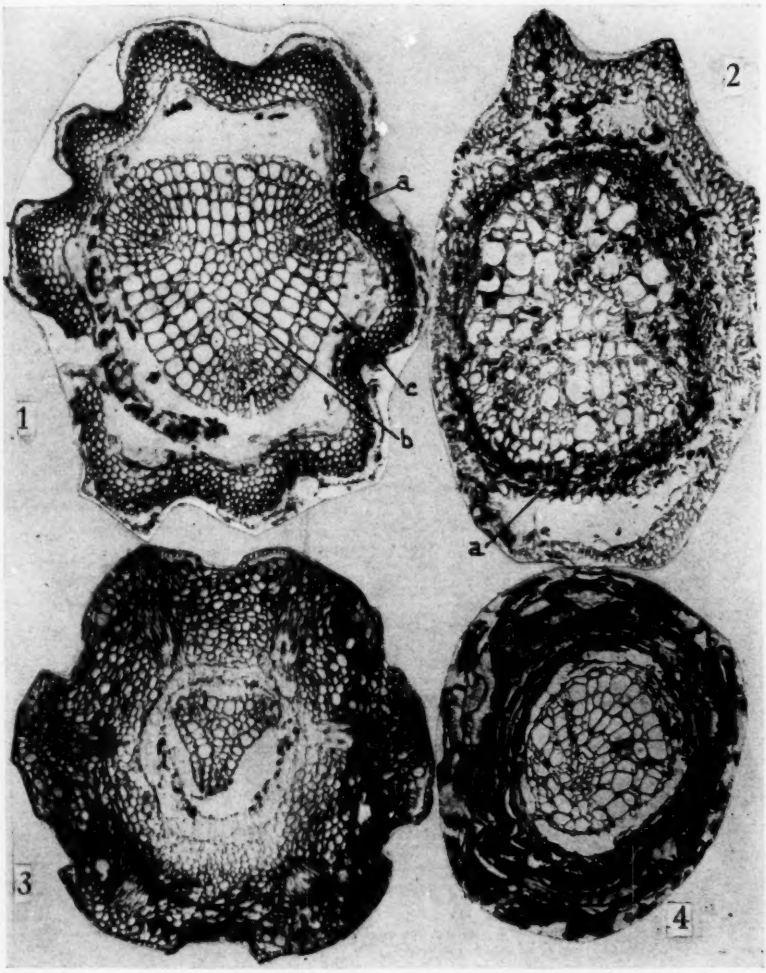
Sphenophyllum plurifoliatum

Fig. 1. Transverse section of stem showing eight cortical furrows: *a*, protoxylem lacuna; *b*, metaxylem; *c*, parenchyma ray extending through three rows of secondary wood. From slide 1523, × 17.

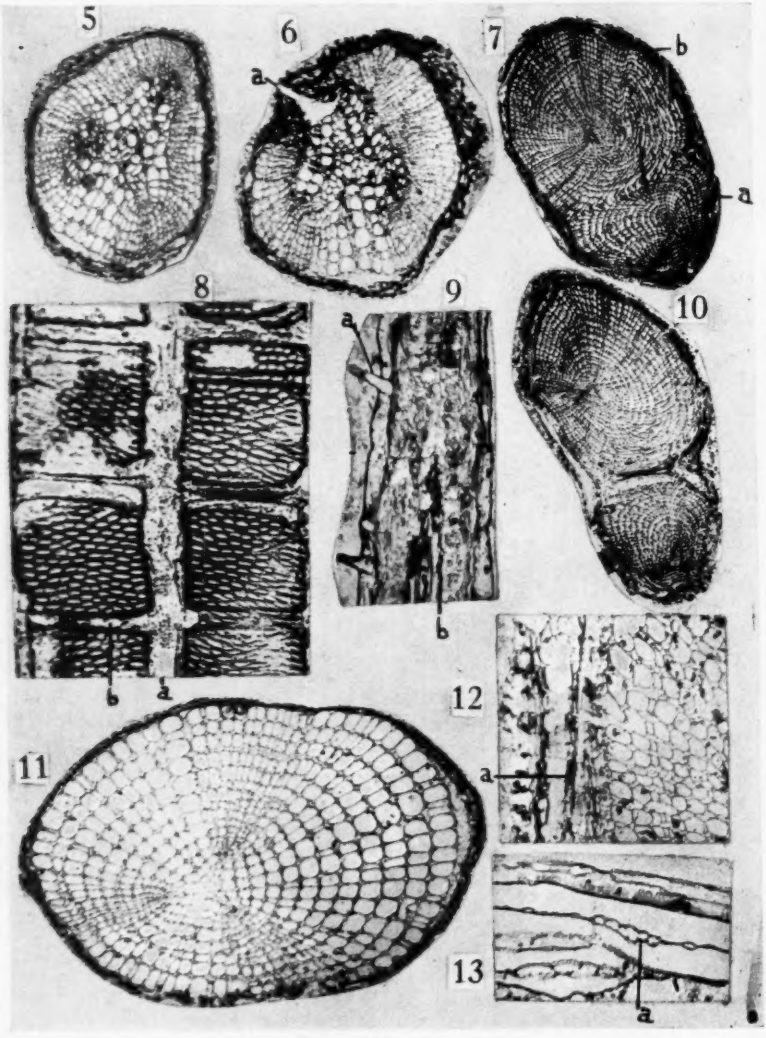
Fig. 2. Transverse section of stem showing sclerotic type of stele: *a*, internal periderm. From slide 1524, × 34.

Fig. 3. Transverse section of a stem at the node showing all six leaf traces passing into the leaf bases. From slide 1525, × 15.

Fig. 4. Transverse section of a medium-sized root showing thick periderm layer. Secondary wood unevenly developed. From slide 1526, × 60.



BAXTER—*SPHENOPHYLLUM PLURIFOLIATUM*



BAXTER—*SPHENOPHYLLUM PLURIFOLIATUM*

Explanation of Plate

PLATE 13

Sphenophyllum plurifoliatum

Fig. 5. Transverse section of stem showing two zones of secondary wood on two sides of the primary wood triangle with normal development on the third side. From slide 1527, × 17.

Fig. 6. Transverse section of same stem as in fig. 5 at a slightly different level showing wounding of the tissues at the point of origin of the second zone of different growth. From slide 1528, × 17.

Fig. 7. Transverse section of a stem showing unequal dichotomy: *a*, main axis; *b*, branch. From slide 1529, × 4.

Fig. 8. Longitudinal-radial section of two rows of inter-fascicular tracheids (vessels?) showing characteristic reticulate pitting: *a*, area occupied by vertical parenchyma cells; *b*, one of horizontal-radiate parenchyma cells. From slide 1530, × 85.

Fig. 9. Longitudinal section of small *Sphenophyllum* root: *a*, root hair; *b*, endodermis. From slide 1526, × 45.

Fig. 10. Same stem as in fig. 7. Forking of stem almost complete; orientation same as above. From slide 1531, × 4.

Fig. 11. Transverse section of a stem with approximately twelve rows of secondary wood radiating out from the central triangular protostele. Note that interfascicular and fascicular wood are identical towards outer margin. Ray structure uniform throughout. From slide 1532, × 11.

Fig. 12. Transverse section of a portion of stem showing interfascicular wood, cambium and two layers of periderm: *a*, inner periderm. From slide 1533, × 22.

Fig. 13. Longitudinal-tangential view of secondary wood: *a*, horizontal-radiate ray approximately six cells deep. From slide 1534, × 60.

Explanation of Plate

PLATE 14

Sphenophyllum plurifoliatum

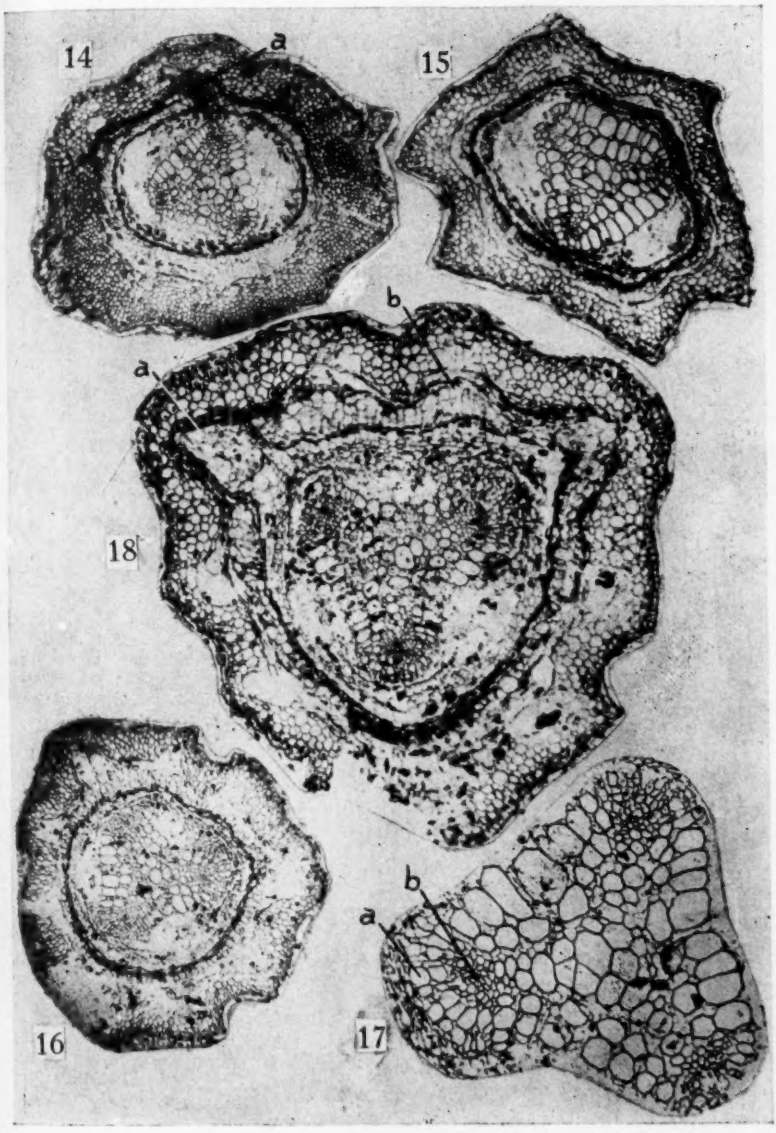
Fig. 14. Transverse section of stem in region of the internode showing production of adventitious root: *a*, adventitious root arising from protoxylem. From slide 1535, × 18.

Fig. 15. Transverse section of same stem as in fig. 14 showing characteristic hexagonal shape. From slide 1536, × 18.

Fig. 16. Same stem as above, with section approaching the node. Note rounded outline and clearly defined cortical furrows opposite interfascicular wood. From slide 1537, × 18.

Fig. 17. Enlargement of the stele of preceding figure: *a*, secondary wood; *b*, protoxylem. From slide 1537, × 60.

Fig. 18. Same stem as above, transverse section at lower edge of the node: *a*, adventitious root preceding branch; *b*, swelling in periderm at position of trace to a side leaf. From slide 1538, × 24.



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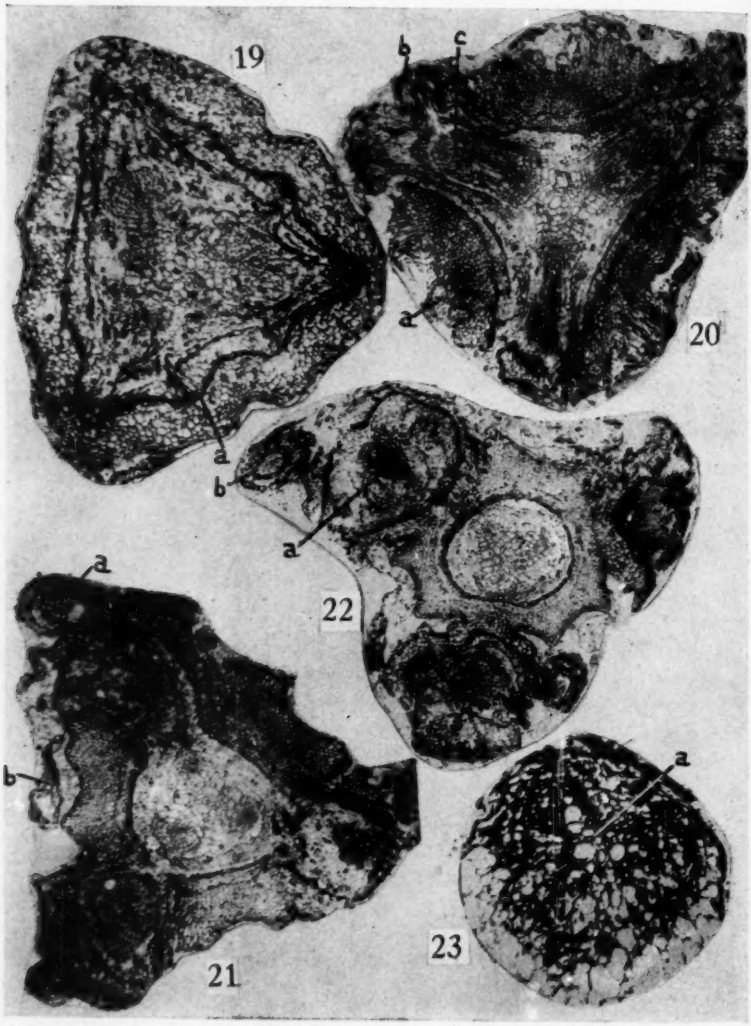


BAXTER—*SPHENOPHYLLUM PLURIFOLIATUM*

Explanation of Plate

PLATE 15

Sphenophyllum plurifoliatum

Fig. 19. Same stem as in fig. 18, showing a section slightly nearer the center of the node: *a*, trace to one of six side leaves. From slide 1539, × 12.

Fig. 20. Continuing nodal series in same stem: *a*, leaf base of one of six side leaves; *b*, adventitious root; *c*, vascular bundle to branch. From slide 1537, × 12.

Fig. 21. Same stem as above; vascular bundles to branches have become separated from main stele: *a*, adventitious root shown in fig. 20 *(b)*: *b*, segment of one of six side leaves. From slide 1540, × 12.

Fig. 22. Continuing nodal series in same stem, showing whorl of three branches almost free from the main stem: *a*, triangular primary wood; *b*, adventitious root. From slide 1541, × 12.

Fig. 23. Enlargement of central portion of one of the three branches at a slightly higher point: *a*, triangular protostele. From slide 1542, × 75.

Explanation of Plate

PLATE 16

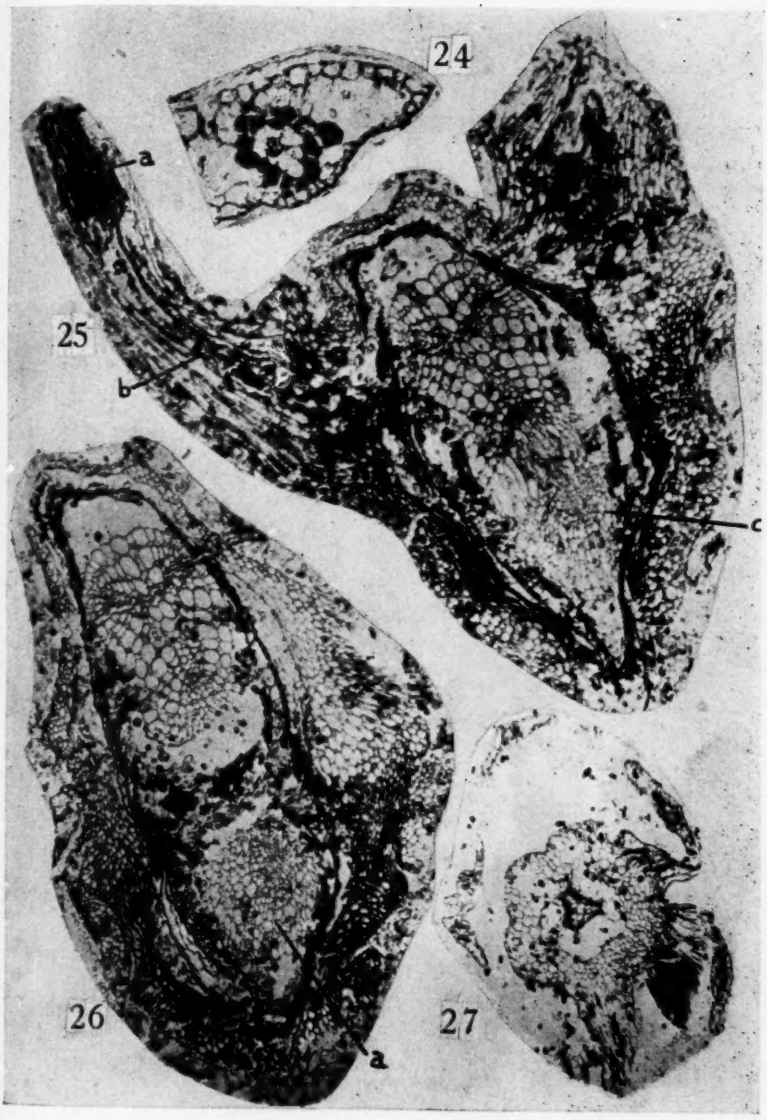
Sphenophyllum plurifoliatum

Fig. 24. Segment of a *Sphenophyllum* leaf showing conspicuous dark fiber ring surrounding small elements of vein. From slide 1543, × 120.

Fig. 25. Transverse section of a stem showing branching: *a*, root branching; *b*, modified adventitious root; *c*, large bundle of fascicular wood coming off into side branch. From slide 1544, × 22.

Fig. 26. Same stem as in fig. 25 at higher point of branch departure: *a*, vascular bundle shown in fig. 25 *(c)* now separated from the central stele. From slide 1545, × 22.

Fig. 27. Transverse section of a small stem at a node with portions of its six leaves surrounding it. From slide 1546, × 26.



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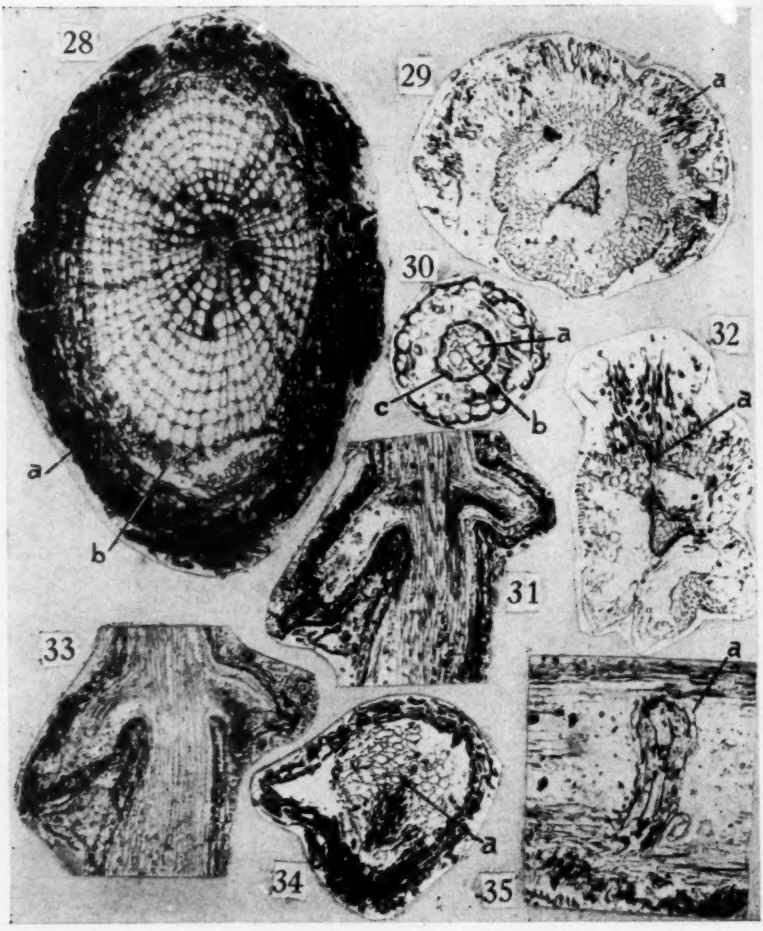

BAXTER—*SPHENOPHYLLUM PLURIFOLIATUM*

Explanation of Plate

PLATE 17

Sphenophyllum plurifoliatum

Fig. 28. Transverse section of a large root: *a*, periderm; *b*, phloem. From slide 1547, × 20.

Fig. 29. Transverse section of a stem at a node showing fused whorl of leaves: *a*, leaf base. From slide 1546, × 24.

Fig. 30. Transverse section of a small root: *a*, secondary xylem; *b*, protoxylem; *c*, endodermis. From slide 1526, × 45.

Fig. 31. Longitudinal section of a stem with large branching roots. Note horizontal passage of vascular tissue into right-hand root. From slide 1548, × 15.

Fig. 32. Transverse section of a small stem at node: *a*, V-shaped leaf traces shown pulled away from the protoxylem angle of the stele. From slide 1546, × 24.

Fig. 33. Same stem as in fig. 31, showing branching of small roots from one of the large roots. From slide 1534, × 15.

Fig. 34. Transverse section of small root showing endogenous origin of branch root: *a*, protoxylem. From slide 1549, × 30.

Fig. 35. Longitudinal section through the middle cortex of a stem showing an adventitious root in both transverse and longitudinal view: *a*, characteristic large epidermal cells. From slide 1550, × 23.

MISCELLANEOUS NEW APOCYNACEAE AND ASCLEPIADACEAE

ROBERT E. WOODSON, JR.

Morleya Woodson, gen. nov. Apocynacearum (Plumerioideae-Plumerieae-Alstoniinae).—Calyx 5-partitus eglanduligerus, lobis aequalibus imbricatis in anthesim caducis. Corolla salverformis, tubo ampulliformi basi staminigero, limbi lobis 5 aestivatione sinistrorso. Antherae 5 subsessiles omnino fertiles compresse ovatae. Ovarii carpella 2 valde subinferiora super receptaculo apocarpa, ovulis in quoque loculo ca. 16, 4-seriatim positis, stigmate sessili doliiformi apiculis 2 minutis erectis minute puberulo-papillatis, nectario nullo. Fructus ignotus.—Arbores. Folia alternata, petiolo supra medio glanduligero. Inflorescentia terminalis thyrsiformis pluriflora, bracteis minimis. Species typicum succedit:

MORLEYA **leipocalyx** Woodson, spec. nov.—Arbor ca. 12 m. alta, ramulis teretibus glabris cortice brunneis. Folia petiolata oblonga vel obovato-oblonga apice valde acuminata basi abrupte decurrentia cum petiolo ad medio glanduligero 10–12 cm. longa 2–3 cm. lata firmiter membranacea glabra. Inflorescentia glabra ramosa foliis brevior, pedicellis ca. 0.5 cm. longis, bracteis minimis caducis. Calycis laciniae ovato-trigonales ca. 0.1 cm. longae minute ciliolatae mox caducae. Corollae albae extus glabrae tubus ampulliformis ca. 1.3 cm. longus basi ca. 0.1 cm. diam. ibique staminiger lobi oblongo-dolabriformes ca. 1.4 cm. longi 0.6 cm. lati patuli. Antherae ca. 0.15 cm. longae. Ovarii carpella glabra vix 0.1 cm. alta; stigmate ca. 0.1 cm. alto. Nomen e λειπο et κάλυξ compositum. —COSTA RICA: Guanacaste: near ridge crest, alt. ca. 200 m.; north of La Cruz on proposed route of Inter-American Highway, 14 miles south of Nicaraguan border, Aug. 13, 1946, *Thomas Morley 770* (Herb. Chicago Nat. Hist. Mus., TYPE).

Morleya suggests relationship to *Plumeria* through its half-inferior ovary, but is distinguished amongst all known Alstoniinae through its peculiar caducous calyx lobes and glandular petioles. I am indebted to Dr. P. C. Standley for calling my attention to this remarkable plant.

MANDEVILLA **longipes** Woodson, spec. nov.—Frutex volubilis ca. 5–7 m. altus ramulis tenuibus ferrugine hirtellis internodiis sat elongatis. Folia opposita petiolata elliptica apice subcaudato-acuminata basi anguste sagittato-cordata 6–9 cm. longa 2–4 cm. lata utrinque puberulo-hirtella supra nervo medio sparse glanduligero petiolo 0.5–1.0 cm. longo. Inflorescentiae axillares alternatae racemiformes pluriflorae pedunculo 8–10 cm. longo sparse minuteque hirtello bracteis lanceolatis 0.2–0.3 cm. longis. Flores ut dicuntur albi medio fulvi; pedicellis elongatis tenuibus 2.0–2.5 cm. longis minute hirtellis; calycis laciniis ovatis acutis ca. 0.2 cm. longis; corollae salverformis vel subsalverformis tubo ca. 3 cm. longo basi ca. 0.1 cm. diam. ostio ca. 0.2 cm. diam. indistincte gibboso paullo supra medio staminigero lobis dolabriformibus ca. 1.5 cm. longis patulis valde contortis. Folliculi valde moniliformes tenues 15–20 cm. longi laeves.—COLOMBIA: BOYACA:

[1] Issued September 20, 1948.

low thin forest, alt. 4500 ft., La Chapon, July 27, 1932, *A. E. Lawrance 361* (Herb. Missouri Bot. Gard., TYPE). At first mistaken for *M. subsagittata*, to which it must be closely related, but differing in its conspicuously elongate, lax (not secund) pedicels. In addition, the flower color of the latter species, as I know it in Panama, is a soft yellow, but with the "brownish yellow center" of which Mr. Lawrance speaks (or possibly more nearly reddish-orange).

MANDEVILLA **turgida** Woodson, spec. nov.—Suffrutex ut videtur suberecta habitu ignota; ramulis crassiusculis cortice irregulariter subalatis rubro-brunneis minute puberulis internodiis ca. 1.0–1.5 cm. longis; foliis oppositis breviter petiolatis ellipticis vel oblongo-ellipticis apice basique acutis 3–4 cm. longis 1.0–1.7 cm. latis rigide coriaceis subenervis illustribus nervo medio supra ad longitudinem sparse glanduligero subtus minute puberulis caeterumque glabris petiolo ca. 0.3 cm. longo; inflorescentiae racemiformes subterminales pluriflores pedunculo ca. 1.5 cm. longo; pedicellis ca. 0.3 cm. longis minute puberulis; bracteis scariaceis minimis; calycis laciniis ovato-lanceolatis anguste acutis ca. 0.15 cm. longis papillatis, intus basi multiglanduligeris; corollae infundibuliformis gilvae extus glabrae tubo proprio ca. 1.5 cm. longo basi ca. 0.15 cm. diam. faucibus anguste campanulatis ca. 1.7 cm. longis ostio ca. 1 cm. diam. lobis oblique obovatis breviter apiculatis ca. 1 cm. longis patulis; antheris oblongo-sagittatis dorso glabris ca. 0.4 cm. longis basi truncate 2-lobatis; carpellis oblongoideis ca. 0.15 cm. longis glabris; nectariis 5 plus minusve concrescentibus ovaria ca. dimidio aequantibus; stigmate umbraculiformi breviter apiculato ca. 0.2 cm. longo; folliculis ignotis. —VENEZUELA: BOLIVAR: Cerro Guaiquinima, Alto Río Paragua, alt. 1740 m., Oct., 1943, *F. Cardona 959* (U. S. Nat. Herb., TYPE).

A most unusual member of the shrubby section of subgen. Exothostemon; allied to the *M. Vanheurckii* complex, of which the old Roraima Shield so abounds, but differing from all in the extremely small, elliptic leaves.

MALOUETIA **Quadricasarum** Woodson, spec. nov.—Arbor ca. 15 m. alta trunco basi ca. 20 cm. diam. Ramuli dichotomi glabri cortice brunneo. Folia opposita lamina late elliptica apice longiuscule subcaudato-acuminata basi late obtusa 10–15 cm. longa 5.0–6.5 cm. lata subcoriacea glabra. Cymae umbelliformes subsessiles pluriflorae. Flores albo-galbini; pedicello ca. 1 cm. longo glabro; calycis laciniis subfoliaceis haud imbricatis apicem versus patulis oblongo-lanceolatis apice obtusis vel rotundatis 0.3–0.35 cm. longis margine sparse ciliatis caeterumque glabris intus basi ad margines 1-squamelligeris; corollae salverformis tubo anguste conico ca. 1.2 cm. longo basi ca. 0.12 cm. diam. ostio ca. 0.05 cm. diam. faucibus staminigeris ibique callosis 5-dentatis extus omnino glabro, lobis patulis inaequilateraliter elliptico-lanceolatis ca. 1 cm. longis extus glabris intus dense pilosis; antheris exsertis ca. 0.25 cm. longis dorso dense pilosis; ovario ca. 0.15 cm. alto dense piloso, nectario annulari; stigmate fusiformi ca. 0.1 cm. longo. —COLOMBIA: El Valle: costa del Pacifico, Río Cajambre, Silva, alt. 5–80 m., May 5–15, 1944, *J. Cuatrecasas 17522* (Herb. Missouri Bot. Gard., TYPE).

In my revision of *Malouetia* (Ann. Missouri Bot. Gard. 22:238. 1935), this species keys to the neighborhood of *M. Schomburgkii* because of its subfoliaceous calyx lobes, although its general appearance is more suggestive of the widespread *A. tamaquarina*.

PRESTONIA **Haughtii** Woodson, spec. nov.—Suffrutex volubilis alte scandens; ramulis crassiusculis glabris bene lenticellatis. Folia opposita breviter petiolata lamina obovato-ovali apice mucronulata basi obtusa vel late acuta 20–30 cm. longa 8–11 cm. lata firmiter membranacea vel subcoriacea glabra supra illustri subtus pallidiori petiolo ca. 1 cm. longo. Inflorescentia terminalis simplex racemiformis ca. 15-flora pedunculo deflexo ca. 8–10 cm. longo glabro bracteis minimis. Flores apicem versus subaggregati; pedicello ca. 1.5 cm. longo inconspicue pilosulo; calycis campanulati coriacei ut videtur plus minusve purpurissati ca. 1.5 cm. longi laciniis ovato-trigonalibus acutis ca. 1 cm. longis indistincte papillatis intus squamellam deltoideam minute erosam instructis; corollae salverformis pallide luteae tubo cylindrico ca. 1.5 cm. longo basi ca. 2 mm. diam. extus glabro, lobis late dolabriformibus ca. 1 cm. longis patentibus, faucium annulo ca. 2 mm. alto obscure 5-lobato albo, appendicibus epistaminalibus vix inclusis ca. 1 mm. longis; antheris vix inclusis ca. 5 mm. longis valde sagittatis glabris; ovariis ca. 1 mm. longis glabris, stigmate non viso, nectariis 5 carnosis basi concrescentibus ca. 2.5 mm. longis. Folliculi ignoti.—COLOMBIA: ANTIOQUIA: edge of forest, alt. under 50 m., Nicocli, June 25, 1946, *O. Haught 4911* (Herb. Missouri Bot. Gard., TYPE).

Closely allied to the following, but differing in the more elongate leaves, somewhat shorter corolla-tube, and nectaries surpassing the ovary.

PRESTONIA **macrophylla** Woodson, spec. nov.—Suffrutex volubilis alte scandens; ramulis crassiusculis glabris valde longitudinaliter striatis et inconspicue lenticellatis. Folia opposita breviuscule petiolata; lamina late obovata apice emarginata basi obtusa vel latissime acuta coriacea glabra supra illustri subtus pallidiori; petiolo ca. 1 cm. longo. Inflorescentia terminalis simplex racemiformis ca. 20-flora; pedunculo 8–10 cm. longo; bracteis minimis. Flores haud specialiter aggregati spiraliter dispositi; pedicello ca. 1 cm. longo sparse appresse pilosulo; calycis basi campanulati coriacei ut videtur plus minusve purpurissati ca. 1.5 cm. longi laciniis oblongo-trigonalibus acuminatis ca. 7 mm. longis extus sparse appresseque pilosulis squamellam 3-angularem ca. 3 mm. longam munitis; corollae salverformis pallide luteae tubo ca. 2 cm. longo basi ca. 4 mm. diam. apicem prope paulo attenuato extus glabro, lobis late dolabriformibus ca. 2.5 cm. longis patulis, faucium annulo ca. 3 mm. alto obscure 3-lobato, appendicibus epistaminalibus inclusis linearibus ca. 3 mm. longis; antheris vix inclusis valde sagittatis 5 mm. longis glabris; ovariis ovoideis glabris ca. 1 mm. longis; nectariis 5 carnosis basi coalitis ca. 2 mm. longis. Folliculi ignoti.—COLOMBIA: ANTIOQUIA: Río Turbo at mouth of Quebrada de los Indios, alt. under 50 m., Turbo, July 15, 1946, *O. Haught 4377* (Herb. Missouri Bot. Gard., TYPE).

At first sight, this species may be mistaken for *P. obovata*, of Panama, from which it differs in the fleshy texture of the floral nectary, typical of the South American representation of the § Annulares. Actually, the closest relative of *P. macrophylla* appears to be the preceding species and *P. didyma*, from which it differs in the obovate leaves, minute bracts, and larger flowers.

FORSTERONIA **propinqua** Woodson, spec. nov.—Suffrutex volubilis gracilis; ramulis gracilibus bene lenticellatis glabratis juventate ferrugineo-pilosulis. Folia opposita breviter petiolata lamina elliptico-oblonga anguste acuminata basi obscurissime cordata 5–8 cm. longa 2–3 cm. lata delicate membranacea opaca supra glabra subtus in axillis nervi medii ferrugineo-barbata nervo medio supra basi pauciglanduligero, petiolo 3–4 mm. longo. Inflorescentia terminalis spicate thyrsiformis multiflora; pedunculo ca. 4–5 cm. longo; pedicellis subnullis. Calycis laciniae ovatae acutae ca. 2 mm. longae extus minute pilosulae esquamelligerae. Corollae rotato-campanulatae extus glabrae gilvae tubus ca. 1 mm. longus faucibus ca. equilatis; lobis ovato-oblongis ca. 2.5 mm. longis patulis; antheris valde exsertis oblongo-panduliformibus basi 2-lobatis ca. 1.5 mm. longis glabris; ovario syncarpo ca. 0.5 mm. longo pilosulo; stigmate umbraculiformi ca. 1.5 mm. longo longe 2-apiculato; nectariis 5 discretis ovarium semiaequantibus. Folliculi ignoti.—COLOMBIA: ANTIOQUIA: forest on Río Guadualito, alt. about 50 m., Turbo, May 1, 1946, *O. Haught 4818* (Herb. Missouri Bot. Gard., TYPE).

This species is remarkable in its syncarpous ovary, which allies it with *F. spicata* of the Caribbean basin of Central and South America and the Antilles, a very common species. From that species, *F. propinqua* may be separated readily by the smaller, oblong-elliptic foliage and strictly terminal inflorescences which are less densely pubescent and with somewhat smaller flowers.

FORSTERONIA **mediocris** Woodson, spec. nov.—Frutex volubilis, ramulis graciliusculis conspicue lenticellatis glabris internodiis sat elongatis. Folia opposita petiolata elliptico-oblonga apice breviuscule subcaudato-acuminata basi rotundata 10–12 cm. longa 4.5–5.5 cm. lata membranacea utrinque glabra, petiolis ca. 0.7 cm. longis. Inflorescentia terminalis anguste thyrsiformis multiflora, pedunculo primario ca. 12 cm. longo minutissime puberulo-papillato ramulis secundariis pluribus basi ca. 2.5 cm. apicem versus gradatim abbreviatis usque 0.1 cm. longis puberulo-papillatis, pedicellis congestis ca. 0.1 cm. longis ut in pedunculis vestitis, bracteis minutis vix bene visis; calycis laciniis late deltoideis acutis 0.1 cm. longis extus minute puberulo-papillatis squamellis nullis; corolla alba campanulata extus intusque minute puberulo-papillata tubo ca. 0.05 mm. longo lobis patulis ovato-ellipticis ca. 0.15 cm. longis; antheris fere plane exsertis 0.08 cm. longis apice pilosulis basi truncatis vix 2-lobatis filamentis liberis; ovario apocarpo ca. 0.05 mm. alto minute pilosulo stigmate incluso ca. 0.05 cm. alto, nectariis 5 integris ovarium aequantibus.—COLOMBIA: CAQUETA: Florencia, entre matorrales residuales de monte, alt. 400 m., Marzo 29, 1940, *J. Cuatrecasas 8800* (U. S. Nat. Herb., TYPE).

This species, the general appearance of which is conveyed by the specific adjective, apparently is closely allied to *F. elachista* Blake and *F. graciloides* Woods., both of which have more diffuse, floriferous inflorescences and roughly obovate leaves; in *F. mediocris*, as well, the flowers are somewhat larger, and the larger anthers more widely exserted.

MATELEA **purpureolineata** Woodson, spec. nov.—Herbae volubiles fere omnino parte trifariam pubescentes pilis et laxe strigulosis et densius minuteque puberulis tum glandularibus tum eglandularibus; ramulis gracilibus internodiis elongatis. Folia opposita longe petiolata ovato-elliptica acuminata cordata sinu lato lobis inflexis 3.5–5.5 cm. longa 2–3 cm. lata membranacea; petiolis tenuibus 2.0–2.5 cm. longis. Inflorescentia alternato-axillaris corymbiformis pluriflora; pedunculo ca. 1 cm. longo; pedicellis ca. 0.5 cm. longis; bracteis minutis. Calycis lobi 5 oblongo-ovati acuminati ca. 0.4 cm. longi extus trifariam pubescentes, squamellis alternatis solitariis ovoideis compressis. Corolla rotato-campanulata gilva venulis 15 purpureis ornata extus pubescens; tubo ca. 0.45 cm. longo ostio ca. 0.3 cm. diam. intus sparse pilosulo prope mediam staminigero; lobis late oblongo-ellipticis apice rotundatis paululo obliquis patulis ca. 0.7 cm. longis. Gynostegium subsessile tubi corollae prope mediam insertum ca. 0.15 cm. diam.; stigmate late umbonato; polliniis horizontalibus latissime reniformibus inaequaliter compressis ca. 0.25 mm. longis, caudiculis subnullis, corpusculo anguste sagittato minuto. Corona annulata lobis 5 latissime 3-angularibus patulis ca. 0.25 mm. longis quibusque processu interiore anguste ligulato ca. 1.25 mm. longo supra gynostegium alte inflexo. Folliculi ignoti.—COLOMBIA: CUNDINAMARCA: hillside east of Apulo, along trail to Anapoima, alt. 460–600 m., thickets, May 4, 1944, *E. P. Killip, A. Dugand & R. Jaramillo 38165* (Herb. Missouri Bot. Gard., TYPE; U. S. Nat. Herb., ISOTYPE).

This species, referable to subgen. *Chthamalia* (cf. Ann. Missouri Bot. Gard. 28:221. 1941), is particularly notable amongst the Mateleas known to me because of the long, narrow, inflexed internal processes of the corona lobes, and the high insertion of the gynostegium, recalling *Gonolobus* subgen. *Pseudolachnostoma*.

A NEW AMSONIA FROM THE TRANS-PECOS

AMSONIA **Tharpii** Woodson, spec. nov.—Herbae perennes suffrutescentes caudice lignoso inveterato ramis herbaceis pluribus 1–2 dm. altis densiuscule canescenti-pilosis. Folia alternato-approximata congesta subsessilia anguste lanceolata acuminata 2.5–4.0 cm. longa 0.2–0.35 cm. lata rarius basi latiuscule elliptica usque 1.2 cm. lata subcoriacea glabra vel nervo medio inferne sparse pilosulo. Inflorescentia terminalis pauciflora. Flores mediocres ut videntur dilute caerulei pedicellis pilosulis ca. 0.3 cm. longis. Calycis laciniae anguste lanceolatae longe acuminatae ca. 0.35 cm. longae subfoliaceae apicem versus pilosulo-barbatae. Corollae subsalverformis extus omnino glabrae tubus 1.4 cm. longus basi ca. 0.1 cm. diam. faucibus intus pilosulis ca. 0.2 cm. diam. lobis anguste ellipticis ca. 0.6 cm. longis patulis. Stamina prope corollae fauces inserta antheris 0.2 cm. longis.

Ovaria oblongoidea glabra ca. 0.15 cm. alta stylo gracili stigmate globoso papillato ca. 0.1 cm. diam. apice obtuse 2-lobato. Folliculi breviusculi crassiusculi subfusiformes continui glabri ca. 2.5–3.5 cm. longi.—TEXAS: PECOS: frequent on limestone hills 21 miles northeast of Ft. Stockton, on McCamey highway, April 19, 1946, *B. H. Warnock 46183* (Herb. Missouri Bot. Gard., TYPE; Herb. Univ. Texas, ISOTYPE); mesa remnant with guayule, June 21, 1943, *B. C. Tharp 43-508* (Herb. Missouri Bot. Gard.; Herb. Univ. Texas).

This rather unattractive, but wholly distinctive, species was first sent to me in the fruiting condition by Dr. Tharp in 1943. Failing to recognize it, I suggested that a look-out for it be kept upon future visits to Pecos County. This spring Mr. Warnock came upon it apparently in considerable numbers and in good flowering condition. Upon first glance at the flowers, I was disgusted to see how closely they resemble those of such ambiguous species as *A. Palmeri, A. birtella* and *A. Peeblesii* in a superficial way. Then, with my specimens of the last-named species before me, I noticed that in all of them the stamens are inserted slightly above midway within the corolla-tube, whilst in Mr. Warnock's plant the stamens are inserted in the upper quarter of the tube, close beneath the orifice. This difference in position of the stamens results in a slightly different constriction of the corolla orifice, as well as corolla throats of somewhat different shape, the throat of *A. Tharpii* being decidedly shorter and more abruptly constricted than in the neighboring species. Thus, I have no further qualms about the description of the novelty, and am pleased to dedicate it to Dr. Tharp who richly merits such recognition. The woody caudex of the type specimen of *A. Tharpii* is very strikingly thickened and lignified, appearing like a dwarfed Japanese tree ("bonsai") with the many stem-bases of past seasons. It must be many years old.

QUANTITATIVE DETERMINATION OF THE PIGMENT CONTENT OF SINGLE CELLS BY MEANS OF A NEW MICROSPECTROPHOTOMETER

BARRY COMMONER

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I. Introduction

Information concerning the physiological activities of single living cells has long been a goal of biological investigation. Analytical biochemistry of tissue masses, while yielding precise chemical data, unfortunately is limited by the fact that it involves procedures tending to disrupt the integrated chemical activities which are the mark of living cells. On the other hand, integrated information can be obtained from studies of whole organisms, organs or tissue fragments, but the data necessarily represent a pooling of the varied rates and directions of chemical activities of at least many thousands of cells.

It has become increasingly clear that these classical methods need to be supplemented by procedures which can produce data referable to individual cells. Thus, for example, investigation of the mechanisms involved in the enormously varied biochemical expression of presumably identical nuclei in the different cells of an organism demands data which can distinguish between biochemical activities of two neighboring cells in a tissue.

Almost the only means of investigating single tissue cells—without removing them from their neighbors and injuring them to the point of death—is light. Because many of the cellular compounds of interest to the biologist have rather characteristic absorption spectra, the determination of the optical density of a living cell in light of various wave-lengths can supply data on the type and amount of certain of these substances present. Since the pioneer investigations of MacMunn (1914), spectroscopy has attracted increasing interest as a means of probing the living cell. Quantitative studies of cellular absorption spectra became possible when several German optical firms developed photographic spectrographs for use with high-power microscopes (see Dhéré, 1933). More recently modern photoelectrical methods have been applied to this type of apparatus by the school of Caspersson (1940) in Sweden, and by Pollister and Ris (1947) in this country. Their work, while chiefly concerned with the determination of nucleic acids and proteins in various parts of the cell, has shown conclusively that microspectrophotometry is technically sound (with proper precautions) and admirably suited to the development of new methods for studying single cell physiology.

The present paper describes a new microspectrophotometer, designed specifically for the study of the biochemical changes which occur in single living cells. As an example of its applicability to this problem, studies of the pigment content of single plant cells are also presented.

II. The Apparatus

The equipment is diagrammed in fig. 1. To simplify the problem of providing a source of monochromatic light throughout the spectrum, a Beckman quartz spectrophotometer is used as a source. This instrument, with its photocell housing and cell-carrier removed, is positioned to direct its light-beam into a vertical microscope. In place of the usual movable substage microscope mirror, a fixed 45-degree first-surface aluminized mirror is mounted on the microscope base to direct the light into the microscope. The microscope and spectrophotometer are rigidly mounted on a common base. To permit the use of the Beckman instrument in its own capacity, the latter is mounted on a sliding track so that it can be moved away from the microscope.

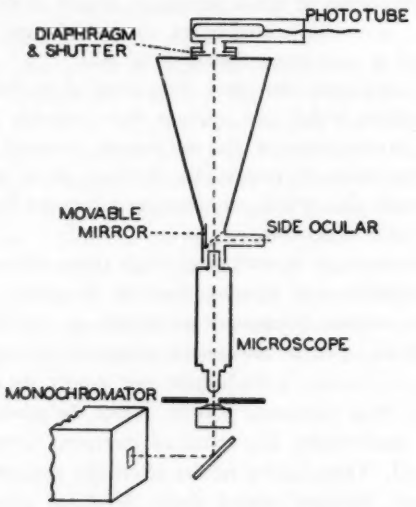

Fig. 1. Optical diagram of microspectrophotometer. Light source is a monochromator from Beckman spectrophotometer. The object may be viewed in the side ocular or scanned by the phototube.

Above the microscope and in line with its optical axis is mounted a modified photomicrographic camera with a side observation ocular. The latter provides a view of the microscope field when a movable 45-degree mirror is thrown across the microscope axis. In place of the camera's plate-holder is a board on which is fastened a large camera shutter and iris diaphragm. The latter is used to determine the area of the image scanned by the photocell, which is mounted directly into the shutter frame. The photocell is of the photomultiplier type, and is used in conjunction with a Photovolt amplifier.

The microscope is equipped with a set of apochromatic lenses and a condenser of N.A. 1.4. The emergent beam of the Beckman instrument is well collimated,

and by proper focusing of the substage condenser critical illumination can be attained. The side observation ocular contains a cross-hair and ocular micrometer. The entire apparatus is aligned so that the object centered in the cross-hair is also centered in the opening of the diaphragm below the photocell. The ocular is further adjusted to bring the object into sharp focus when its image lies in the plane of the photocell. In this way it is possible to select by the side ocular the precise area to be scanned by the photocell. By rotating the ocular mirror, the light path can then proceed to the photocell and the measurement can be made.

The instrument is operated as follows: The wave-length dial of the spectrophotometer is set at the desired wave-length. The object is brought into view in the side ocular and the size of the area to be studied is determined on the ocular scale. The upper diaphragm is then set to this size. The slide is moved slightly so that the cross-hairs fall on a clear area just next to the object (mounted in water). The mirror is then thrown out of position and the apparatus adjusted to yield a full-scale deflection on the photocell galvanometer. This can be done by altering either the slit-width of the spectrophotometer, the opening of the substage diaphragm (within small limits), or the sensitivity of the amplifier. The ocular mirror is brought into position again and the slide moved to bring the desired area of the study object into the cross-hair point. The mirror is immediately thrown out again. The galvanometer then reads the transmission of the object as per cent of the incident light which emerges. The procedure is then repeated at a new wave-length and so on until the desired spectral range has been examined. In practice it is possible to make a single reading in about 30–40 seconds.

The equipment functions within the range 340–800 mμ, and its lower limit is due only to the lack of quartz optics in the microscope. A minimum object area of 4 μ can be studied. The amount of light incident on the object area is so small that no heat damage has been observed. *Tradescantia* staminal hair cells, for example, continue to show active protoplasmic streaming at the end of the determination of a complete absorption spectrum.

III. Optical Accuracy

The entire apparatus was tested for optical accuracy by determining the absorption spectrum of a sample of crystalline cyanin in 1 per cent HCl (concentration: 6.64 mgm. per liter). The solution was placed in a quartz micro-absorption cell, 1 cm. thick, and a matched cell was filled with pure solvent. Both cells were sealed and placed side by side (on a clean slide) on the microscope stage with their optical faces horizontal. The 40$\times$ objective was focused on the upper surface of the solvent cell, and the stage coordinates noted. A corresponding position for the test cell was also determined and coordinates noted. The transmission of the sample was then determined by setting the galvanometer scale to 100 with the solvent in focus and then moving to the noted area of the sample cell. In this way the absorption spectrum of the cyanin sample was obtained in the region of its characteristic visible absorption band. This completed, the cells were removed

from the glass slide and placed in the regular Beckman cell carrier in the usual way. The spectrophotometer was reassembled and the absorption spectrum of the sample redetermined in the Beckman instrument itself.

The two sets of data obtained from the same sample are presented in fig. 2. It will be noted that the spectral agreement is complete, the sample yielding its characteristic absorption peak of 510 mμ on both instruments. Thus the microspectrophotometer seems to be accurate with regard to the spectral bands which impinge on the object at various positions of the Beckman wave-length scale. Furthermore, it will be noted that the optical density of the sample at its absorption peak is

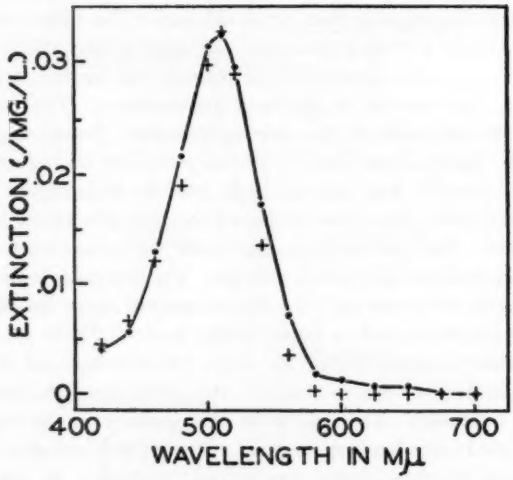

Fig. 2. Absorption spectra of the same sample of cyanin (6.64 mg. per liter in 1 0/0 HCl) obtained by the microspectrophotometer (solid line) and a Beckman spectrophotometer (points indicated by +).

practically identical for both sets of data (extinction = .317 on the microspectrophotometer; extinction = .318 on the Beckman instrument). This indicates that the apparatus is accurate with regard to intensity measurements, and that its response is adequately linear. It will be noted that there is a small discrepancy between the two absorption spectra at the limits of the cyanin band. This may be due to the fact that the slit widths used in the micro determination (1.0–2.0 mm.) were twice those used on the Beckman instrument. This was necessitated by the excessive light loss caused by the thick cell and does not occur in ordinary measurements which can be made at slit widths of .1–.7 mm.

IV. Determination of Pigments in Single Coleus Hair-cells

Coleus is a convenient plant for the study of the physiology of pigment production. Its many varieties produce a very wide range of anthocyanin concentrations, and cells of varying degrees of pigmentation frequently can be obtained from the same plant. The studies reported below were initiated as a preliminary step in the investigation of the biochemical effects of virus infections which produce strong alterations in the degree of pigmentation of *Coleus*.

Pigment production in this plant is chiefly localized in the epidermal cells of the leaves. Of these cells, those comprising the hairs covering the leaf's upper surface are best suited to optical measurements. Because of their regular shape (truncated cones), the thickness of any given part of such a cell can be determined by measuring its diameter at that point. The regular shape also permits easy

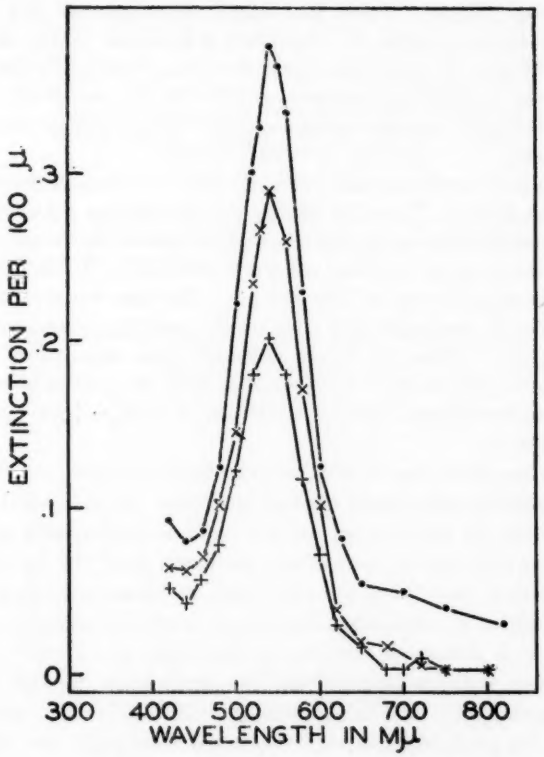

Fig. 3. Absorption spectra of three different epidermal cells from a *Coleus* leaf. The upper curve is for a flat epidermal cell, the center curve for the second cell of an epidermal hair, and the lower curve for the basal cell of the same hair.

calculation of cell volume. Finally, the varying diameter provides a ready means of testing the effect of thickness on optical density.

Epidermal hairs stripped from the midrib of mature leaves and mounted in distilled water under a thin cover glass were examined to locate cells not overlaid by any other tissue and oriented in a plane horizontal to the microscope's optical axis. By means of the ocular disc mounted in the side ocular, the cells' dimensions were determined. By reference to the same scale a point in the center of each cell was located. All measurements of optical density were made on these points. The absorption spectra of the selected cells were determined by the procedure outlined above.

Typical results of such measurements are shown in fig. 3. For the sake of uniformity the data are presented in terms of the optical density per 100 μ since the thickness of the cell at the point scanned will obviously alter the light transmission values. Figure 3 shows that reliable determinations of a characteristic band at 540 mμ can be obtained. According to Robinson (1931) the red color of the leaves of *Coleus Blumei* is due to an anthocyanin, cyanin. Purified preparations of cyanin have an absorption maximum at 510–520 mμ (see below), and it seemed likely therefore that this substance is responsible for the peak at 540 mμ exhibited by *Coleus* cells.

As a means of confirming this point, the effect of alkali on the cell absorption spectrum was studied. Cyanin in alkaline solution develops a deep blue color, and the absorption band is correspondingly shifted toward the longer wave-lengths. The absorption spectrum of a hair cell was determined in the usual way in the entire available spectral range of 340–800 mμ. The slide was then flooded with a dilute solution of ammonium hydroxide which caused the normal red color of the cells to give way to blue, and the absorption spectrum redetermined. The results, shown in fig. 4, indicate that the expected shift in the position of the visible absorption band does indeed occur. The addition of alkali shifts the peak from 540 mμ to 600 mμ.

Figure 4 also shows that in addition to the peak in visible light these cells have a second absorption maximum in the near-ultraviolet. In the normal cell this peak is at 360 mμ; in the alkali-treated cell it is broadened considerably with the maximum at about 380–390 mμ. It had been previously noted (by Mr. Milton Zucker of this laboratory) that *Coleus* hair-cells which are low in anthocyanin turn bright yellow on addition of alkali, suggesting that a flavone glucoside was present. All flavones have an absorption maximum in the region of 320–330 mμ, which in alkaline solution shifts to about 380 mμ. It seemed likely therefore that the cells' absorption maximum at 360 mμ represented flavone rather than anthocyanin.

To test this possibility, spectra were obtained from single hair cells of a green variety of *Coleus*, which shows no red color and appears to be free of anthocyanin. A series of spectra made on single hair cells from this type of plant revealed in each case an absorption maximum at 360 mμ. Typical results are shown in fig. 5. In

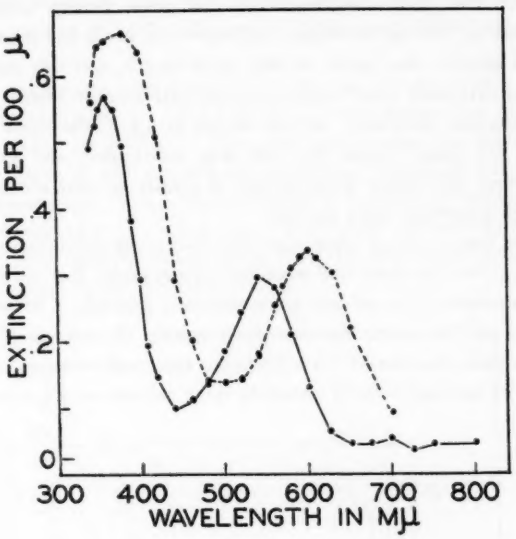

Fig. 4. The absorption spectrum for a hair cell (basal) from a red *Coleus* leaf in water (solid line) and ammonium hydroxide (dotted line).

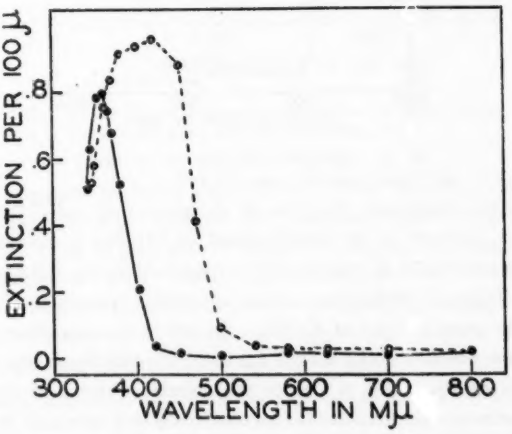

Fig. 5. The absorption spectrum of a hair cell (basal) from a green *Coleus* leaf in water (solid line) and ammonium hydroxide (broken line).

agreement with the gross observation that the green variety lacks anthocyanin, no obvious peak at 540 mμ is visible. (However, a small and possibly significant rise in optical density does occur at that wave-length, and this may indicate the presence of an extremely small amount of the anthocyanin which occurs in large quantities in the red varieties.) As also shown in fig. 5, the effect of ammonium hydroxide on the green *Coleus* hair cell was determined, and the shift of the maximum toward the longer wave-lengths is similar to that obtained in the red variety, though somewhat more marked.

From these data it seems clear that the absorption maximum of *Coleus* hair cells located at 360 mμ does not represent anthocyanin, but rather is due to a constituent common to the red and green varieties, probably a flavone. To obtain additional data on this point, the absorption spectra of extracts of both red and green varieties were determined (in a Beckman spectrophotometer). The extracts were prepared by boiling leaves in relatively large volumes of 1 per cent HCl.

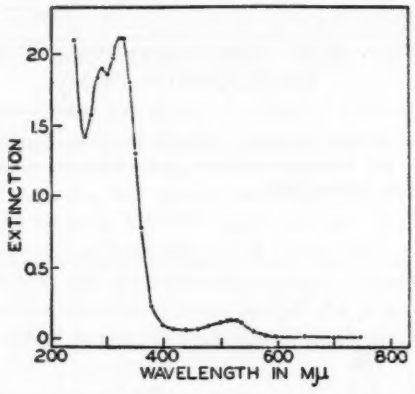

Fig. 6. Absorption spectrum of an extract of red *Coleus* leaves in 1 per cent HCl.

Figure 6 is the absorption spectrum of the extract of red *Coleus* leaves (in 1 per cent HCl). A band in the neighborhood of 520 mμ is obvious, but the absorption in the ultraviolet is considerably stronger, showing definite peaks at 290 and 325 mμ. Figure 7 (solid line) is the absorption spectrum of the extract of leaves from the green variety of *Coleus*. At pH 2 no absorption occurs in the visible range, but the two peaks at 290 mμ and 325–330 mμ in the ultraviolet are similar to those shown in fig. 6. Since the colorless extract of the green leaves lacks anthocyanin and yet exhibits the two ultraviolet maxima, it is again obvious that these maxima are not due to anthocyanin. That they may result from a flavone is again indicated by the second curve (dotted line) in fig. 7, which represents the absorption spectrum of the extract at an alkaline pH (8.4). The

maximum is shifted toward the longer wave lengths. Such alkaline solutions of the green *Coleus* extract show the yellow color characteristic of flavones at alkaline pH.

To separate the constituent pigments of the red *Coleus* extract the original solution was extracted with an equal volume of ethyl acetate (in which flavones but not anthocyanins are soluble). The ethyl acetate fraction was then shaken with phosphate buffer of pH 6.4 and the ultraviolet absorption spectrum of the aqueous layer determined. This is shown in fig. 8, and it is at once apparent that the constituent removed by the ethyl acetate does show the two peaks at 290 and 320 mμ. The buffer solution, upon being made alkaline, turns yellow and then brown, and so appears to contain a flavone.

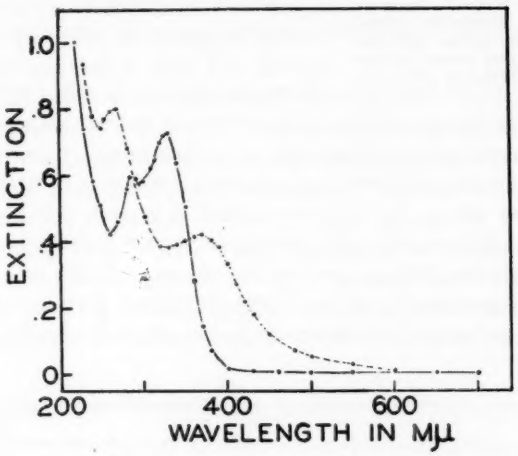

Fig. 7. Absorption spectrum of an extract of green *Coleus* leaves at pH 2 (solid line) and pH 8.4 (broken line).

These results suggest therefore that the red *Coleus* epidermal cells contain, in addition to anthocyanin, other material with absorption maxima (in acid solution) at 290 and 320–325 mμ, at least part of which is a flavone. This material is present in the cells of the green variety which lack anthocyanin.[1]

With this information at hand it is possible to reexamine the significance of the two absorption maxima obtained from intact living cells. In the first place, it will be noted that the anthocyanin maximum of the extract is at 520 mμ, while the maximum

[1]Further extraction experiments suggest that the peaks at 290 mμ and 320 mμ may be due to different substances. Repeated extractions of leaf preparations with ethyl acetate cause a progressive reduction in the 320 mμ peak of the original extract, but much of the 290 mμ absorption remains in the aqueous fraction. It is possible therefore that the 290 mμ peak is due to the ultraviolet absorption of anthocyanin (crystalline cyanin has a peak at 275–280 mμ). Furthermore, this peak may in part be due to the presence of a leucoanthocyanin, since recent reports (Fogel, 1948) suggest that these substances are characterized by absorption maxima at this wave-length.

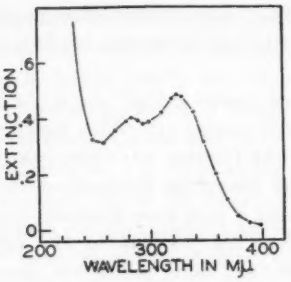

Fig. 8. Absorption spectrum of material removed from red *Coleus* extract by ethyl acetate. The sample was obtained by shaking the original extract with ethyl acetate and then extracting the ethyl acetate fraction with phosphate buffer of pH 6.4.

obtained in the living cell is at 540 mμ. This discrepancy has been confirmed repeatedly. The maximum of the extracted anthocyanin is not altered by purification procedures such as repeated extraction with ethyl acetate or lead acetate precipitation. Paper chromatograms of the extract yield only a single red-colored band, and this preparation also has a peak at 520 mμ. It seems reasonable to conclude therefore that the anthocyanin absorption represents a single substance which in solution (at acid pH) has a peak at 520 mμ. Since all of a considerable number of spectra of living cells yield a maximum at 540 mμ it is necessary to conclude that the *Coleus* anthocyanin within the cell is in some state other than the one found in solution. The acidity of the cell might seem to offer an explanation of this phenomenon. It can be seen from fig. 9 that the extract at pH 8.4 has a maximum at 590 mμ rather than 520 mμ, and it might be expected that the shift to 540 mμ shown by the cell may represent a pH effect. While this explanation cannot as yet be ruled out there is some evidence against it. Repeated attempts to shift the cell's peak to 520 mμ by flooding the cell with acetic acid have failed. Furthermore, even the peak at extremely alkaline pH is not the same in the cell (600 mμ) and extract (590 mμ). It seems possible therefore that the shift of

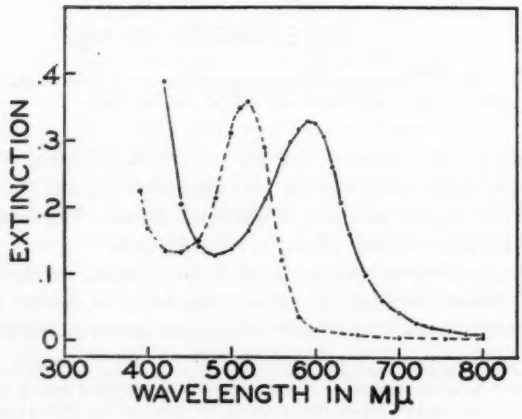

Fig. 9. The absorption spectrum of an extract of red *Coleus* leaves at pH 2 (broken line) and pH 8.4 (solid line).

absorption maximum characteristic of the cell may represent some effect other than that of pH. One possible explanation is that the anthocyanin in the cell is combined with some other cellular constituent, such as a protein, a phenomenon which frequently alters a pigment's molecular structure sufficiently to cause a significant shift in absorption maxima.

A similar discrepancy between the maxima of the extracts and the intact cell exists in the case of the non-anthocyanin absorption bands. The living cells have an absorption maximum at 360 mμ which shifts to 400–425 mμ in alkali; the extract's maximum at 320 mμ shifts to 370 mμ in alkali. In both cases the response to alkali is similar, suggesting that the substance responsible for the 320 mμ peak in the extracts accounts for the 360 mμ peak in the living cell. Again, this would suggest that the substance responsible for this absorption band—probably a flavone—occurs in the cell in some form of combination which does not exist in extract.

The possibility that the flavone and anthocyanin pigments in the living cell occur in a complex together with some other cellular constituent may have an important bearing on further analysis of the metabolic role of these substances.

From the evidence presented above it seems reasonable to conclude that the absorption maximum observed in living *Coleus* hair cells at 540 mμ which shifts to 600 mμ in an alkaline medium represents anthocyanin, and that the maximum found at 360 mμ which shifts to 420–425 mμ in alkali probably represents a flavone compound. It is therefore possible to use these facts as a means of making quantitative determinations of both anthocyanin and flavone content of single hair cells.

V. Determinations of Anthocyanin and Flavone Contents

The considerations necessary to effect accurate determinations of the concentrations of substances present in cells from measurements of the optical density are expressed by the Beer-Lambert laws of light absorption. These state that the amount of light energy absorbed is a function of the number of molecules (which specifically absorb light of the given wave-length) contained in the path of the light beam. Since each layer of the sample reduces the amount of light impinging on subsequent layers of the sample this relationship is logarithmic and is expressed by the equation:

$$E = \log_{10} 1/T = kcd$$

where E is the extinction or optical density; T is the fraction of incident light which emerges from the sample; c is the concentration of the specifically absorbing substance per unit volume of the sample; d is the thickness of the sample; k is a constant which expresses the tendency of the molecules of the specific substance to absorb impinging light energy of the given wave-length.

In the procedures described above, the optical measurement made is of T, while d is determined by measuring the thickness of the cell at the point scanned.

It follows from the above equation that the concentration of the substance present in the sample is proportional to the optical density per unit thickness, and that the ratio of the concentrations of two different samples is equal to the ratio of their respective E/d quotients. However, this relationship holds only when all of the light absorption is due to the presence of the specific substance in question. In ordinary spectrophotometric practice non-specific light losses are accounted for by comparing the sample with a "blank" which is identical with the sample except that it lacks the specific substance. This procedure cannot be followed in determinations of substances naturally occurring in a living cell and indirect methods must be used.

In general, two such methods can be applied: (1) The cell's non-specific light absorption may be determined at a wave-length outside the characteristic absorption band of the substance in question. It must then be assumed that this amount of non-specific light-loss also occurs at the wave-length of the substance's absorption maximum. This assumption may not be valid, particularly if the extrapolation is made into the ultraviolet range. In the case of the present measurements, however, it would seem relatively safe to assume, for example, that the non-specific light loss at the anthocyanin maximum (540 mμ) is equal to the light absorption in red light where the cell (since it is free of chlorophyll) contains little specifically absorbing material. The validity of applying this assumption to the absorption at 360 mμ is more doubtful.

(2) A second method is the conversion of the substance in question into a new molecular form in which it exhibits an absorption maximum at a new wave-length. Thus, addition of alkali shifts the flavone absorption band in the cell from 360 mμ to about 400–420 mμ. Hence the cell's optical density at 420 mμ can be measured at a normal pH and again after addition of ammonia. The difference between the two values of E is then an accurate measure of the relative flavone concentration of the cell. This method can also be applied to the determination of anthocyanin by measuring the cell's extinction value at 625 mμ before and after addition of alkali. The data presented below are intended to examine the relative validity of both methods as a means of determining anthocyanin and flavone concentrations in single cells.

In the first instance it is necessary to determine whether the total optical density of the cell is indeed a linear function of the thickness of the area scanned. This problem was examined by making use of the conical shape of the *Coleus* hair cells. Assuming that the anthocyanin concentration is uniform throughout the entire volume of the cell, it is possible to study the effect of cell thickness by taking absorption readings at various points along the length of a single cell, thereby obtaining a graded series of thicknesses through a sample of identical pigment concentration. If the test object follows the Beer-Lambert laws, the plot of extinction versus thickness should follow a straight line. Several

typical sets of such measurements are shown in fig. 10, and it is clear that the data do conform to the expected optical relationship.

To test the relative validity of the methods outlined above, the following determinations were made on nine *Coleus* (red) hair cells selected at random from strips of midrib epidermis.

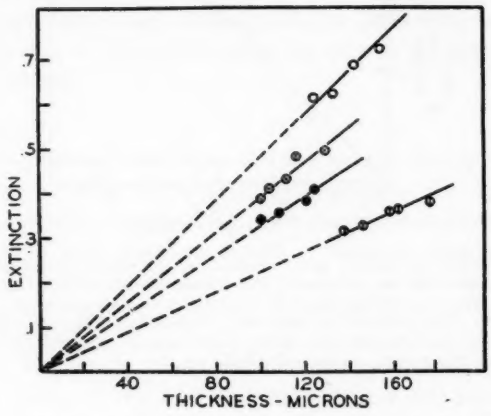

Fig. 10. The extinction (at 540 mμ) of each of four different red *Coleus* hair cells at various thicknesses. Since the cells are conical in shape, areas of different thicknesses could be scanned by focusing on various points along the cells' long axes.

With the cells mounted in water, the value of E per 100 μ of cell thickness was determined at wave-lengths 700 mμ, 625 mμ, 540 mμ, 440 mμ, 420 mμ, and 360 mμ. The slide was then flooded with ammonium hydroxide and the E per 100μ values for the same cells determined at 625 mμ and 420 mμ.

From these data the relative anthocyanin concentration of each cell was calculated by the two methods:

(1) Relative anthocyanin concentration $= E_{540} - E_{700}$.

(2) Relative anthocyanin concentration $= E_{625}$ (ammonia) $- E_{625}$.

The relative flavone concentrations were also calculated by the alternative methods:

(1) Relative flavone concentration $= E_{360} - E_{440}$.

(2) Relative flavone concentration $= E_{420}$ (ammonia) $- E_{420}$.

The two sets of values obtained for the relative anthocyanin concentration of the cells are plotted against each other in fig. 11. It is quite clear that both procedures give the same value for the anthocyanin content and it seems valid to use the extinction value at 700 mμ as a measure of the non-specific absorption at 540 mμ.

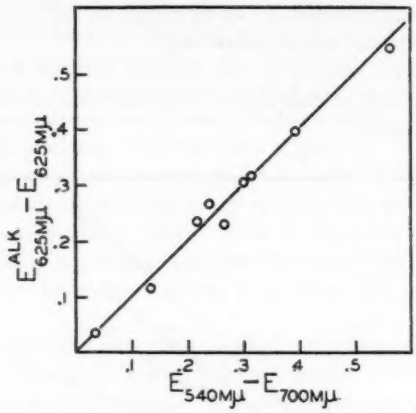

Fig. 11. The relationship between values obtained for the relative anthocyanin concentrations of nine hair cells by two different procedures. The abscissa represents relative anthocyanin concentrations determined from the difference between the cells' extinction values (in water medium) at 700 mμ and 540 mμ. The ordinate represents the corresponding values obtained by determining the difference between the same cells' extinction at 625 mμ in ammonium hydroxide and their extinction at 625 mμ before being treated with alkali.

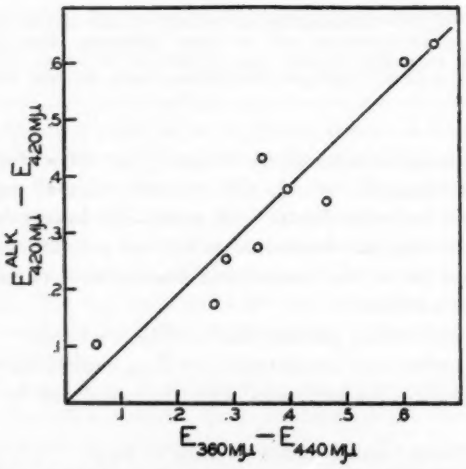

Fig. 12. The relationship between the flavone concentration of nine *Coleus* hair cells determined by two different procedures. The abscissa represents the relative flavone concentrations determined from the difference of the cells' extinction (in water medium) at 360 mμ and 440 mμ. The ordinate represents the corresponding values determined from the difference between the same cells' extinction at 420 mμ in ammonium hydroxide and their extinction at 420 mμ before being treated with alkali.

A similar plot of the flavone data is shown in fig. 12. These points show a significant amount of scatter from the line of proportionality, a result expected from the fact that the measurements were made in the near ultraviolet. Apparently there is a significant amount of random non-specific light loss at this wavelength, and the reliability of the 440 mμ extinctions as a measure of non-specific absorption at 420 mμ is lower than that for the corresponding treatment of the anthocyanin data. Hence while determinations of anthocyanin content may be made without recourse to alkaline treatment, the use of a pH shift seems necessary for flavone estimations.

VI. Discussion

The evidence presented above shows that it is possible, by use of rapid spectrophotometric measurements, to identify and determine the concentration of anthocyanin and flavone in single cells. These results can undoubtedly be applied to determinations of other substances as well. Preliminary experiments show that chloroplast pigments may be identified and estimated by the same procedure.

Furthermore, the method is applicable to determinations of metabolic rates in single cells. Thus the rate of decolorization of methylene blue can be followed in a single cell, and the activity of cellular dehydrogenases thereby determined. Preliminary measurements of this kind made on epidermal cells show that accurate determinations of rates of dehydrogenation can be made within an hour. Corresponding measurements have been made with tetrazolium chloride, a substance which forms a red formazan as a result of dehydrogenase activity.

Such quantitative determinations of various metabolically important substances and rates of reactions in single cells can yield valuable data on questions of cellular differentiation and physiological genetics, and should be applicable to other types of investigation as well.

VII. Summary

(1) A microspectrophotometer of relatively simple construction is described.

(2) The instrument is capable of determining absorption spectra of 4 μ areas of single cells over the spectral range of 340–800 mμ. Its accuracy relative to macro determinations with a Beckman spectrophotometer is demonstrated.

(3) The presence of anthocyanin and flavone in single hair cells of *Coleus* has been demonstrated by such absorption spectra. The absorption maxima of these substances in the living cell is different from those of the substances in solution. It is suggested that this discrepancy may indicate that these substances are combined with some other cellular constituent *in vivo*.

(4) Methods for quantitative determination of anthocyanin and flavone in single cells are presented. Anthocyanin concentrations may be determined in living untreated cells. Flavone estimations are more accurate if determined from the effect of alkali on the substance's absorption maximum.

(5) The microspectrophotometer offers many possibilities of measuring significant metabolic rates in single cells.

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THE SOUTHERN DENT CORNS

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The older maize varieties of the southern United States are of interest for two reasons: (1) Some of them were extensively used in developing the more highly derived varieties of the United States corn belt. (2) They show a strong affinity to the dent corns of central Mexico. When their history can be worked out in more detail, it should shed significant light on the relationships of the brilliant civilizations of central Mexico and the lands to the north.

In the states east of the Mississippi, maize has had, on the whole, a relatively simple history. As we have demonstrated (Brown & Anderson, 1947), the northern flints were widely distributed in pre-Columbian times in the northeastern states where they were the only type of corn grown over a considerable area. In the Gulf States and spreading northward from them, there were at least two other major types of maize: (1) the old white dents, and (2) the Caribbean flints. We do not yet have any exact knowledge of when the intermingling of these southern types began. We do know that by the early nineteenth century the old southern dents were, on the one hand, being intentionally crossed with the Caribbean flints and, on the other hand, with the northern flints. From the latter union there was eventually developed the distinctive, cylindrical dent corn of the United States corn belt.

Today in the southern states one may still find authentic samples of such old dent varieties as Gourdseed and Shoepeg. They are not easy to come by and require extensive searching among conservative families in more or less isolated neighborhoods. Along with them are more modern varieties derived from crosses with the northern flints, with Caribbean flints, and with corn-belt varieties from farther north. For the purpose of this study we have made a rough grouping of the material under observation as: (1) old southern dents, (2) derived southern dents. Our collections of these corns were reasonably complete and our survey is a comprehensive one, particularly in relation to the role played by these varieties in the development of the maize of the United States corn belt. The Caribbean flints, although undoubtedly involved, were peripheral to the area covered by our studies and have therefore been omitted from this survey. These tropical flints, because of their wide distribution in both hemispheres, deserve exhaustive analysis, but to be carried on effectively such a study would require adequate experimental fields in a subtropical environment.

The methods used in this study were essentially those applied to previous surveys of maize in the United States and Latin America. A few selections were

grown and observed over a period of years. In the winter of 1946-47 an intensive effort was made to get together as representative a collection as possible, including large field samples, to show the range of variation. The collection was grown in duplicate at Gray Summit, Missouri, and at Johnston, Iowa. For each culture, photographs were made of representative plants, of representative tassels, ears, and kernels. The variation of the tassel was observed and recorded in detail, and the chromosome knob number was determined from pachytene smears. Much of this information is presented below in tabular summaries.

HISTORY

Compared to the northern flints, the history and archaeology of the southern dents are in a very unsatisfactory state. There are several clear descriptions of the northern flints in the pre-colonial and colonial literature, and over a wide area in the eastern states they are the only archaeological type which has yet been discovered. The dents are a variable lot. We would need to have many more archaeological specimens if we were to do equally as good a job with them as with the northern flints, and as yet we have almost none. From those specimens which we have been able to examine, it is certain that dented varieties were grown in the Great Plains in pre-historic and proto-historic times. The story of corn in that area is apparently a very complicated one. Not until the archaeology of that region is better understood and not until we have seen many more collections which include maize remains will we be in a position to discuss the early history of dent corns in the region now occupied by the United States. For the states east of the Mississippi where we have descriptions of strongly dented varieties in early colonial times, we have as yet seen no archaeological material. As far south as Alabama and Georgia the archaeological record (away from the Mississippi Valley) is made up of wide-seeded flint or flour corns of the same general type as the "northern flints" of New England and Canada. This suggests that the dented varieties described in the Colonial records were relative newcomers and were in the process of pushing northward and eastward at the time of European contact.

Apparently the earliest description of a southern dent corn is in Beverly's history of Virginia written in 1705. He wrote that it is: "a larger grain and looks shriveled with a dent on the back of the grain as if it had never come to perfection; and this they call She corn." In the agricultural note books of Charles Read of New Jersey occurs the earliest reference we have been able to find to dent corn described as such. In an entry for 1756 he lists the weights of various kinds of corn, among others, "Egg-Harbor Dented" and "the long-grained, Lower County corn." (See Woodward, 1941).

At about this same period we have a fairly good description of a deeply dented white corn from Louisiana. Dumont, in his *Mémoires historiques sur la Louisiane*, published in 1753, has the following description (pp. 32–34):

"On distingue deux sortes de mahi, dont l'un est propre à faire de la farine, & l'autre non: ce dernier a le grain tout rond; l'autre l'a un peu plus plat, & se distingue par une espéce de coup d'ongle ou de rainure qui regne sur toute la longeur des graines." [Two kinds of maize can be distinguished, one good to make meal, the other not. The latter has the kernel quite round, the other is a little more flat and is distinguished by a kind of claw point or groove prevailing along the whole length of the kernel.]

John Lorain, whose shrewd observations on maize and maize breeding were unsurpassed until long after his time, provides us with the first detailed description of gourdseed varieties. In a letter dated October 25, 1813, and published in the *Memoirs of the Philadelphia Society for Promoting Agriculture* (Vol. III, pp. 308–310) he described Gourdseed:

"The cob of this is neither so long or thick as the large solid corns but the grains are very long, forming a compact, round and gradual taper to a point where they join the cob. It is vastly more productive than any other known original corn but ripens late and the grains are too soft and open for exportation, unless kiln dried. This variety, so far as my observation goes, is invariably white; for although I have frequently heard of a solid yellow gourdseed corn, yet on investigation, nothing more has appeared than a mixture of the hard yellow corns with the white gourd seed."

In his book on agriculture published posthumously in 1825 Lorain goes into greater detail. He describes the gourdseed varieties as having up to 32 and sometimes even 36 rows of kernels. The results to be obtained from mixing gourdseeds and northern flints were accurately described in considerable detail and the benefits of such a mixture were clearly set forth.

Lorain's writings clearly indicate that the purposeful (as well as accidental) mixing of gourdseeds and flints was already well under way in the early 1800's. From the agricultural press and such early scientific agencies as the U. S. Patent Office and the State Agricultural Reports, one can reconstruct quite accurately the history of the dent varieties of the United States corn belt. Some of the main evidence has already been reviewed in our survey of the northern flint corns and need not be repeated here. We can summarize the results, in so far as the United States corn belt is concerned, by saying that the northern flints and southern dents (originally two very different types of maize) were so repeatedly crossed and recrossed that the mixtures bred from them eventually dominated the entire region. Today the 8-rowed flints are grown, if at all, only in the extreme north, and the gourdseeds and shoepegs have completely disappeared from the actual corn belt. Their very names have been largely forgotten, and even in the southern states it is only a few conservative families who still grow them.

Cytology

A cytological peculiarity of maize is that at certain points on the chromosomes there may be definite knobs of more deeply staining material. The knob number is constant for any individual plant and in the corn of the United States may vary from 0 to about 14 (haploid number). It can therefore be used as one criterion in determining the relationships of various kinds of maize.

The number of chromosome knobs was determined for each of the varieties included in this study. Knob counts were, without exception, made from temporary smears of pachytene chromosomes stained either with aceto-carmine or propionic-carmine. Since the seed from which our cultures were grown was from open-pollinated stocks, they exhibited considerable morphological and cytological variability, as might be expected. For purely physical reasons, where several cultures are involved, it is impracticable to determine knob numbers of a large number of plants of each culture. The data on knob numbers reported herein were taken from two to four plants of each variety and show primarily the overall range of variability between varieties. Had we worked with larger numbers of plants we possibly would have encountered a greater degree of variation within some varieties than is reported.

As is often true, some difficulty was encountered in distinguishing between large chromomeres and small knobs. Our policy has been to count as knobs only those pycnotic enlargements that are strikingly larger than the average chromomere. This practice has been followed even when the enlargement was located at a known knob position. For this reason our counts should be taken as conservative. Since the organizer knob on chromosome No. 6 is present in all strains of maize, we have excluded it in our enumerations of knob number.

It will be noted that knob numbers in this material range from 4 to 12. When the varieties are separated into (1) old southern dents, and (2) derived southern dents, it is immediately apparent that the majority of the high knob varieties are to be found among the first group while those with lower numbers are mostly distributed among the derived southern dents (fig. 1). This association is to be

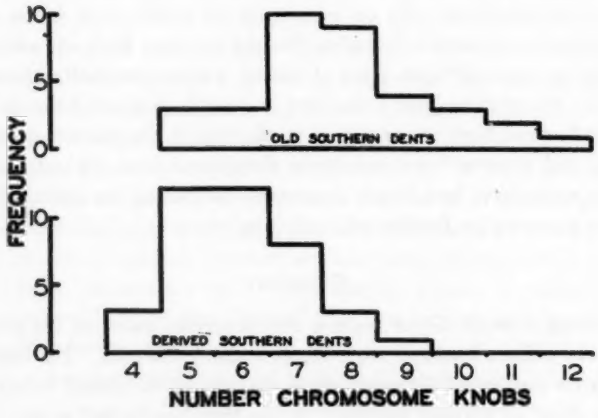

Fig. 1. Distribution of chromosome knobs in southern dent corns.

expected when we take into consideration the history of these two groups of corns. The old southern dents have evolved without drastic change from varieties of central Mexico which are known to possess relatively high numbers of chromosome knobs, while those types we have termed derived southern dents have largely arisen, either at first or second hand, out of crosses between old southern dents and northern varieties with lower knob numbers.

Morphology

The southern dents differ from the northern flints and from corn-belt dent varieties by a number of gross morphological characters, of which the following are more obvious. Plants of most southern varieties are unusually tall as compared to other United States corns. This is true whether we study them in the south or grow them farther to the north. The increased height is due to the presence of more nodes and not to an increase in internode length. In fact, most southern dents resemble Mexican dents in having extremely short internodes above the ear as compared to the long upper internodes of the northern flints (figs. 2–3). Ears are carried high on the culms and are enveloped in tight, thick husks which often extend well beyond the ends of the ears. Husks are usually composed solely of modified leaf sheaths, the blade portion of the husk being only slightly developed, if at all, and as a result one never finds the extensive "flag leaves" that are so common in northern flints. As a group the southern dents do not have tillers, although there are strains, particularly among the derived sorts, that occasionally produce a few. Prop-roots are well developed, and in certain of the more Mexican-like varieties they may be found even at the sixth and seventh nodes when they are grown in the north.

In contrast to the northern flints and most corn-belt maize, the tassels of the southern dents are many-branched and often highly condensed[1] (pls. 20–21). In many varieties the secondary branches are upright and comparatively short, resulting in a "whisk broom" appearance that is rarely found in the northern flints. In general, the numbers of tertiary branches in the tassels of southern dents are much greater than in the northern flints or corn-belt dents.

Most southern dents have a white endosperm although one or two varieties in our collection had yellow. According to the best historical evidence, the older southern dents were all white and the occurrence of yellow endosperm in the more modern strains is the result of outcrossing to yellow varieties. Although cob color is predominantly white, a few exceptional varieties with red cobs were represented in our cultures.

As mentioned above, we have rather arbitrarily divided our collection into (1) old southern dents, and (2) derived southern dents. In the second group we

[1] We are here referring to "condensation" in the technical sense, as defined by Anderson (1944); a condensation (or telescoping) of successive internodes on the tassel branches.

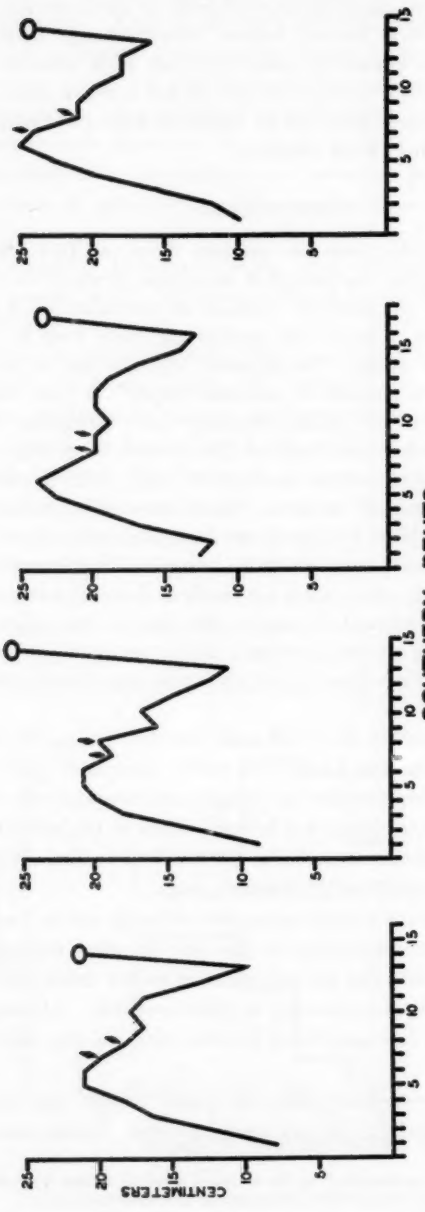

Fig. 2. Internode diagrams of typical individuals of four varieties of southern dent corns. Note the presence of many short internodes above the ears as contrasted to the long upper internodes of the northern flints (fig. 3).

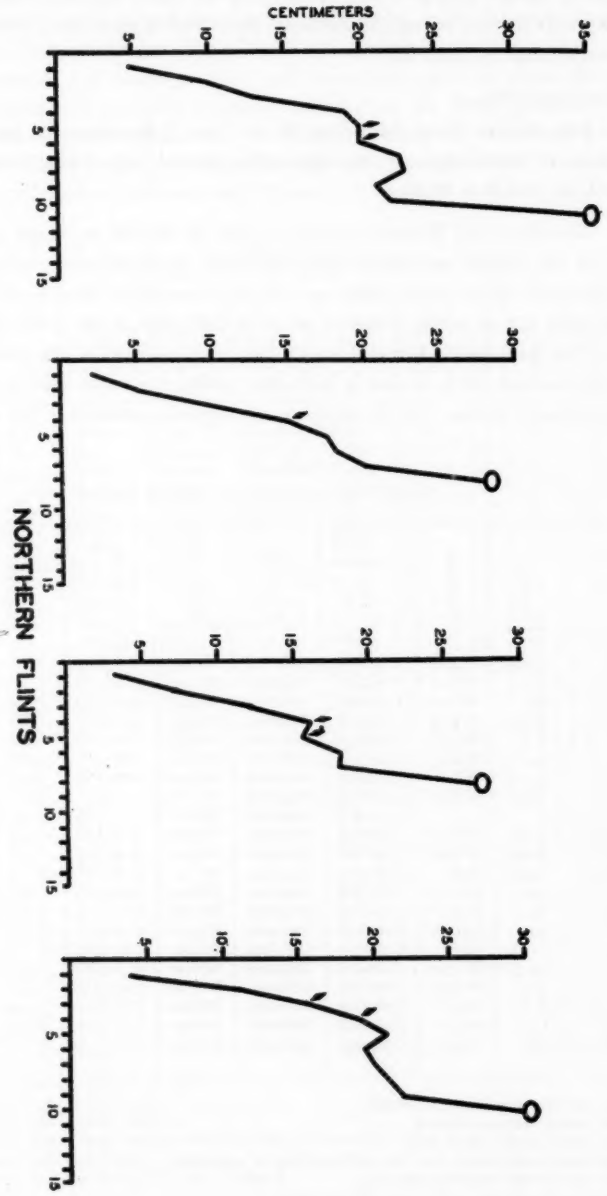

Fig. 3. Internode diagrams of typical individuals of four varieties of northern flint corns.

have placed those varieties whose morphology or whose known history indicates that they were derived by mixing southern dents with Creole flints, northern flints, or varieties from the corn belt.

Old Southern Dents.—

The old southern dents themselves do not form a homogeneous group. Three main types are represented, each one apparently derived from similar varieties which were and are grown in Mexico.

A. *Gourdseed and Shoepeg.*—These names are applied to rough white dents, which, on the whole, are much alike and may represent two extremes of the pointed-kerneled dent corns which are widely known in Mexico as *"pepitillo."* Both of them are so strongly dented as to be collapsed at the pointed tip of the kernel. The gourdseed's kernel, though long and pointed, is also flattened with somewhat rounded sides, so that it looks not unlike the white seed of a cucurbit, hence its popular name. The shoepegs are the opposite extreme. The seed is very

TABLE I
VARIETIES OF OLD SOUTHERN DENTS

Variety	Source	Cob Color	Pericarp	Aleurone	Endosperm	Kernel width (mm.)	K. width/ K. th. (mm.)	Denting*	Mean row number	Number chromosome knobs
Gourdseed	Texas	White	Colorless	Colorless	White	7.1	2.3	5	18.1	6,7,8
Hickory King	Va.	White	Colorless	Colorless	White	13.0	3.4	3	8.4	7
Hickory King	Ga.	White	Colorless	Colorless	White	12.3	3.4	2	8.0	6,11,12
Hickory King	Tenn.	White	Colorless	Colorless	White	12.5	3.5	3	8.0	5,7,8
Jellicorse	Va.	White	Colorless	Colorless	White	7.6	2.1	4	12.7	9
Jane Corn	La.	White	Colorless	Colorless	White	7.0	2.1	2	14.2	10,11
Mexican June	Tenn.	White	Colorless	Colorless	White	8.0	2.2	2	13.8	7,8
Mexican June	Tenn.	White	Colorless	Purple & colorless	White	7.7	2.2	2	14.6	9
Old White Dent (1)	Ark.	White	Colorless	Colorless	White	7.8	2.2	4	16.0	5
Old White Dent (2)	Ark.	White	Colorless	Colorless	White	8.2	2.5	3	15.5	5
Red Cob Chisholm	Texas	Red	Colorless	Colorless	White	9.0	1.8	3	14.3	10
Shoepeg	La.	Red	Colorless	Colorless	White	5.0	1.5	5	18.3	7,8
Shoepeg	La.	Red	Colorless	Colorless	White	6.2	1.7	5	20.0	6
Shoepeg	La.	Red	Colorless	Colorless	White	5.2	1.6	5	18.6	7
Tenn. Red Cob	Tenn.	Red	Colorless	Colorless	White	7.1	2.2	4	14.4	7,8
Tuxpan	La.	White	Colorless	Colorless	White	7.2	2.1	3	14.8	7,8,9
Tuxpan	Va.	White	Colorless	Colorless	White	7.0	2.2	3	14.2	8
White Dent	Ark.	Red	Colorless	Colorless	White	7.0	2.6	4	16.0	7
Yellow Shoepeg	La.	Red	Colorless	Colorless	Yellow	5.2	1.6	5	19.6	7,8,9,10
Yellow Tuxpan	La.	White	Colorless	Colorless	Yellow	8.0	2.1	2	14.0	8

* 0—No soft starch at apex of kernel.
1—Soft starch but no denting.
2—Soft starch and a small dent.
3—Soft starch and a deep dent but no wrinkling of pericarp.
4—Soft starch and wrinkling pericarp.
5—Soft starch and the apex of kernel collapsed.

long and narrow, the sides being almost parallel, and it is pointed at the tip, the point itself usually being turned toward the apex of the ear by the tight husks to form a distinct hook at the top of the kernel (pl. 21).

The gourdseeds and shoepegs may well have been selected from the same fundamental stock and probably represent extremes of the same gourdseed type. The names, however, have been used to distinguish them for many years. The measurements (Table I) and accompanying photographs (pls. 20, 21) are drawn from samples collected in Louisiana and Texas.

TABLE II
VARIETIES OF DERIVED SOUTHERN DENTS

Variety	Source	Cob Color	Pericarp	Aleurone	Endosperm	Kernel width (mm.)	K. width/ K. th. (mm.)	Denting*	Mean row number	Number chromosome knobs
Cambren	Ky.	White	Colorless	Colorless	White	8.0	2.1	3	10.0	6
Caraway's Prolific	La.	Red	Colorless	Colorless	Yellow	6.5	1.9	3	14.3	6,7
Cherokee	Ga.	White	Colorless	Colorless	White	6.7	2.1	3	15.3	5
Clark's Yel. Dent	Texas	Red	Colorless	Colorless	Yellow	8.4	2.5	4	14.4	5,6
Columbia Beauty	Tenn.	Red	Colorless	Colorless	White	6.9	2.2	4	16.8	6
Garretts 1	Ky.	Red	Colorless	Colorless	White	7.0	2.1	3	15.1	5,6
Garretts 2	Ky.	Red	Colorless	Colorless	White	7.2	2.2	3	15.3	7
Giant Yel. Dent	Texas	Red	Colorless	Colorless	Yellow	9.7	2.7	4	12.5	5
Huffman	Tenn.	White	Colorless	Colorless	White	7.5	2.0	3	16.0	5
Jarvis Gol. Prolific	Tenn.	White	Colorless	Colorless	Yellow	7.2	2.0	2	14.0	5
Jarvis Gol. Prolific	Miss.	White	Colorless	Colorless	Yellow	7.3	2.1	2	12.7	4
Johnston Co. White	Mo.	White	Colorless	Colorless	White	7.7	2.0	5	18.0	7
Latham's Double	Va.	Red	Colorless	Colorless	White	5.8	1.8	3	14.8	6
Mammoth Ensilage	Va.	White	Colorless	Colorless	White	8.0	2.3	4	14.5	5
Mosby's Prolific	Va.	White	Colorless	Colorless	White	6.8	2.1	4	16.1	7,8
Mosby's Prolific	Miss.	White	Colorless	Colorless	White	7.0	2.2	2	13.5	5
Mosby's Prolific	La.	White	Colorless	Colorless	White	6.7	2.0	3	12.2	7
Mosby's Prolific	Tenn.	White	Colorless	Colorless	White	6.9	2.1	3	13.7	6
Neal's Paymaster	Ark.	White	Colorless	Colorless	White	6.8	2.1	4	15.6	6
Neal's Paymaster	Miss.	Red	Colorless	Colorless	White	7.4	2.2	4	14.7	6
Sherman	Tenn.	White	Colorless	Colorless	White	6.5	2.1	3	14.8	5
Southern Ill. 1	Ill.	White	Colorless	Colorless	White	9.0	2.6	3	13.6	5
Southern Ill. 2	Ill.	Red& White	Colorless	Colorless	White	7.0	1.5	3	15.3	6,7
Southern S'flake	Va.	White	Colorless	Colorless	White	6.6	2.2	3	17.0	5
Southern Yel. Dent	Miss.	Red	Light yel.	Colorless	Yellow	7.1	2.1	3	16.0	4,5
Strawberry	Texas	Red& White	Var'gated	Colorless	Yellow	7.9	2.4	3	14.6	8,9
Va. Horsetooth	Va.	White	Colorless	Colorless	White	6.2	1.8	3	17.7	4,6,7
Whatley's Prolific	Va.	Red	Colorless	Colorless	White	7.4	1.7	3	15.1	7,8
Yellow Dent	La.	White	Colorless	Colorless	Yellow	8.1	2.5	3	11.1	6
10 Row Dent	Ark.	Red	Colorless	Colorless	White	7.8	2.2	3	13.2	5

* 0. No soft starch at apex of kernel.
1. Soft starch but no denting.
2. Soft starch and a small dent.
3. Soft starch and a deep dent but no wrinkling of pericarp.
4. Soft starch and wrinkling pericarp.
5. Soft starch and the apex of kernel collapsed.

B. *Mexican June Complex.*—This category includes a rather closely related group of corns, the better known of which bear such varietial names as Mexican June, June Corn, Jellicorse, and Tuxpan. Although considerable variation exists within and between varieties, the group may be characterized and distinguished from the gourdseeds and shoepegs by having fewer rows of kernels and a lower degree of denting. Row numbers are usually 12 to 14. Ear shape varies from almost cylindrical to a very strong taper. The base of the ear is frequently compressed, and there is a tendency for slight irregularity in rowing at the base. The kernels are usually longer than wide and are slightly pointed. There is always a distinct cap of soft starch at the tip of the seed, bearing a small to medium dimple dent. Cobs are usually white.

This group of varieties is as Mexican-like as any in our collection. In ear type they are very similar to collections from central Mexico that have been classified by Anderson (1946) as intermediates between Mexican Pyramidal and Mexican Narrow Ear. These varieties likewise show a strong affinity in plant type to certain central Mexican corns. Tassel branches are several and are usually rather short. Leaves are broad and the sheaths often carry strong plant color. The leaf sheaths tightly envelop the culms as contrasted to the loose "puffy" sheaths of the gourdseeds and shoepegs. In the variety Tuxpan, the sheaths above the ears exhibit considerable pubescence both on the backs as well as at the edges, a common characteristic of most varieties of Central Mexico.

C. *Hickory King.*—Hickory King, one of the older varieties of southern dents, possesses a group of ear characteristics that are so distinctive as to make it necessary to place it in a separate category among southern corns. Ears are narrow and cylindrical with 8 to 10 rows of seeds that are often as wide as long. Seeds are strongly flattened on top, and there is a very strong tendency for row pairing. A distinct cap of soft starch with a rather shallow dent and some wrinkling is characteristic of the variety. Plant type in Hickory King is not particularly distinctive. It is similar to many other old southern dents in that the plants are tall, have few tillers, and increasingly short internodes above the ear.

The relationship between Hickory King and certain Mexican corns seems quite clear. There is little doubt that the variety has arisen from a similar group of corns in Mexico that are known as "*tabloncillo.*" They form the commonest varieties of field corn over large areas in western Mexico and belong to the general race of corn termed Mexican Narrow Ear by Anderson (1946).

Derived Southern Dents.—

These corns apparently originated out of mixtures of gourdseeds, shoepegs, semi-hard Mexican June types, tropical flints and cylindrical dents from the corn belt. In ear type some show considerable gourdseed tendency, while others (including many of the "prolifics") appear to be rather closely related to the Mexican June complex. Jarvis Golden Prolific and similar varieties have many

characters in common with Creole or Caribbean flints and may carry rather large amounts of germ-plasm from this or some closely related source. Also among the varieties that we have included with the derived southern dents are those strains which are very similar to corn-belt dents. Perhaps they arose in the southern periphery of the corn belt in the same manner as did most corn-belt corn (i. e. through the union of northern flints and old southern dents) or perhaps they are the result of mixing old or derived southern dents with corn-belt dents.

Importance of the Old Southern Dents for the United States Corn Belt

The old southern dents should be of more than passing interest to corn breeders in that middle-western area centering in Iowa which is known as the United States Corn Belt. In the last few years there has been considerable interest among some United States corn-breeders in the possibility of obtaining superior germ-plasm from maize varieties in Central America and Mexico. Since the old southern dents are so similar to several Mexican types, many of the desired genes might be obtained with far less trouble from certain southern dents than from the Mexican varieties from which they are derived. The fact that they have already been moved part way towards the North should simplify the task of incorporating any of their desired characteristics into corn-belt inbreds. Our preliminary results indicate that the maturity of most southern dents will permit their being used in crosses in the Midwest without resorting to the use of day-length control. Since the general growth habit of southern United States varieties is already fairly similar to northern ones, there probably would be fewer undesirable combinations to be discarded in breeding from southern material.

One specific quality which might prove useful in future breeding programs is the soft texture of the southern dents. Preliminary results indicate that crosses with these southern varieties will produce softer-textured dents than have been available in the corn belt. If changes in the methods of harvesting corn-belt maize should make it desirable to breed for varieties with two or more ears, certain southern varieties of prolific habit could supply the genes necessary for the expression of this character. There are already indications in our data that much of the heterosis in United States corn-belt varieties comes from combining northern flints and the southern dents. It is improbable that the maximum number of genes making for hybrid vigor has already been extracted from these two stocks. It might be possible to increase the potential hybrid vigor of our corn-belt hybrids by bringing deliberately into our inbreds additional sets of differing genes from the northern flints and the southern dents.

One of the most promising uses of the southern dents may be to illuminate the genetics of multiple-factor characters in maize. From the viewpoint either of the practical breeder or of general evolutionary theory, the genes which control multiple-factor differences are of far greater importance than the single genes

ordinarily employed in genetic experiments. Yet in spite of their over-all importance we know little about them, and experiments designed to tell us more have been so discouragingly difficult that little real advance has been made since East's preliminary investigations. A study of the northern flints and southern dents is a promising avenue of approach to this problem. It has been shown from data on species crosses, as well as from theoretical deductions, that in crossing well-differentiated races, all the multiple-factor characters are partly linked in the second generation and that the total effect of this linkage can be removed only by many generations of controlled breeding, if at all. We may therefore expect the multiple-factor characters which differentiate the northern flints from the southern dents still to be somewhat linked in the maize of the United States corn belt. The experience of practical breeders indicates that this expectation is certainly realized. High-row numbers, tapering ears, soft texture, and pointed kernels are characters which went together into corn-belt corn from the southern dents. It is common experience among corn-breeders that this complex of characters still tends to stay together after a century of breeding and selection.

A careful study of the southern dents, particularly in their contrasts to the northern flints, should help us by suggesting character combinations which may still be more or less associated in modern corn-belt varieties. Furthermore, by using corn belt inbreds, northern flints, and southern dents, in controlled experiments, it should eventually be possible to learn what kinds of genes differentiate these types of maize, roughly how many of them there are, and on what chromosomes they are distributed.

Summary

1. The dent corns of the South are of importance because some of them were extensively used during the nineteenth century in developing the more highly derived varieties of the United States corn belt.

2. After several years of preliminary study, a comprehensive collection was assembled and grown in duplicate in Iowa and Missouri. Standardized photographs were made of plants, tassels, and ears. Pachytene smears of the pollen mother cells were studied to determine the number of chromosome knobs. A portion of this information is summarized in tabular form.

3. For the purposes of this discussion the southern dents are roughly grouped into: (1) the old southern dents, and (2) derived southern dents. The former were almost certainly derived from certain Mexican varieties. The derived dents originated from crosses between the old southern dents and northern and tropical flints as well as from crosses with corn-belt dents.

4. No archaeological records of old southern dents or of similar varieties have yet been obtained from the eastern states. Our earliest records are accounts from Louisiana and Virginia in the eighteenth century. The historical evidence for their having been used in the creation of the varieties of the present United

States corn belt is well documented and reasonably complete.

5. The chromosome knob numbers of the old southern dents in our cultures ranged from 4 to 12. As might be expected, numbers were higher among the old southern dents than among the derived dents.

6. The morphology of the southern dent corns is briefly summarized. The more important varieties such as Gourdseed, Shoepeg, and Hickory King, are discussed in more detail.

7. The probable usefulness of the southern dents to practical plant breeding and to theoretical genetics is discussed. It is suggested that they might be more useful sources for certain desirable characters, such as soft texture, than some of the Mexican and Central American varieties which have been considered. To theoretical genetics, they offer a combination of multiple-factor characters which is greatly different from that found in modern United States corn and still more radically different from that of the northern flints. By intercrossing these three types of corn (and with the use of marker genes and cytological analyses) it should be possible to estimate the numbers of multiple-factor genes involved and their distribution in the germ-plasm.

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Explanation of Plates

PLATES 18–23

Representative specimens of "old" and "derived" varieties of southern dent corns; 1—plant; 2—mature tassel; 3—typical ears; 4—seeds. Each division on the background of plant and tassel photographs represents 50 (cm.). Each division on the scale opposite the ears represents 1 (cm.).

Plate 18. Caraway's' Prolific.

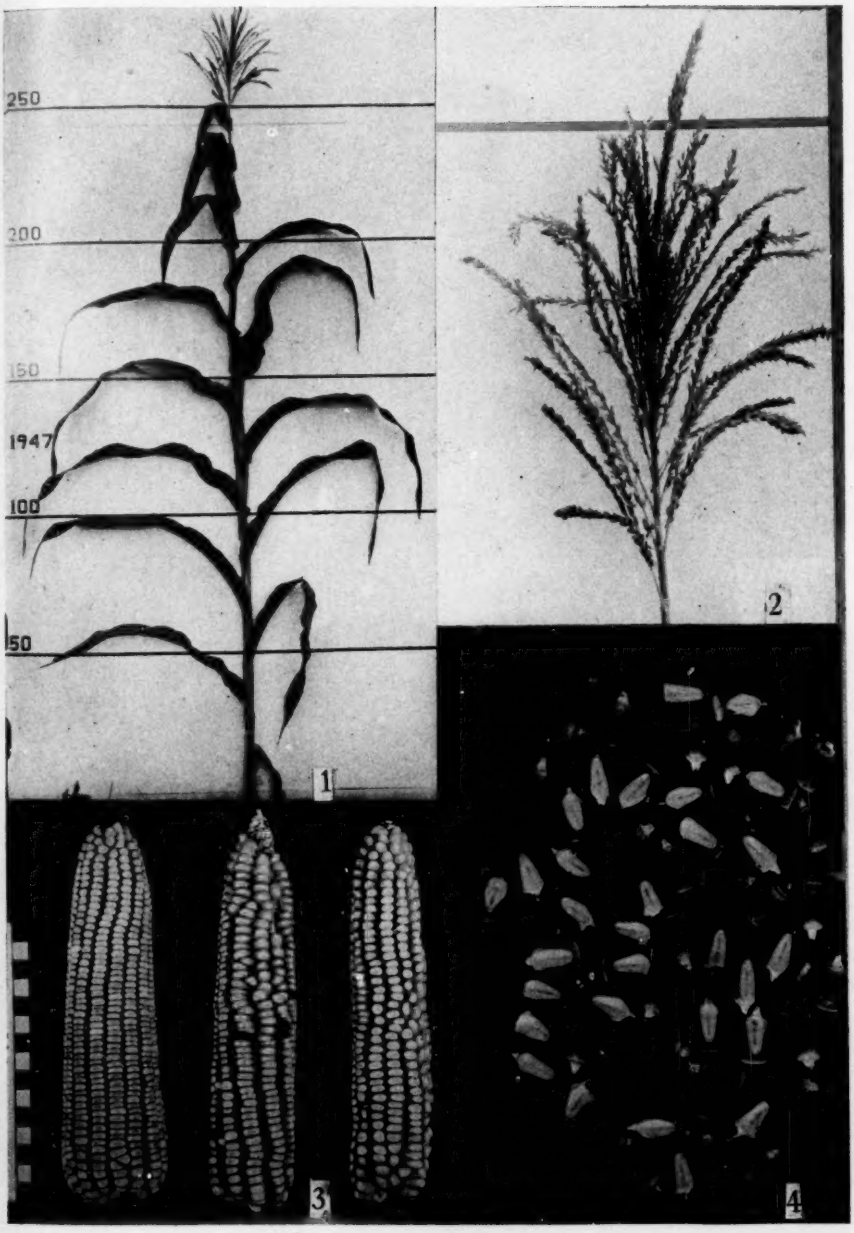
Plate 19. Tuxpan.

Plate 20. Gourdseed.

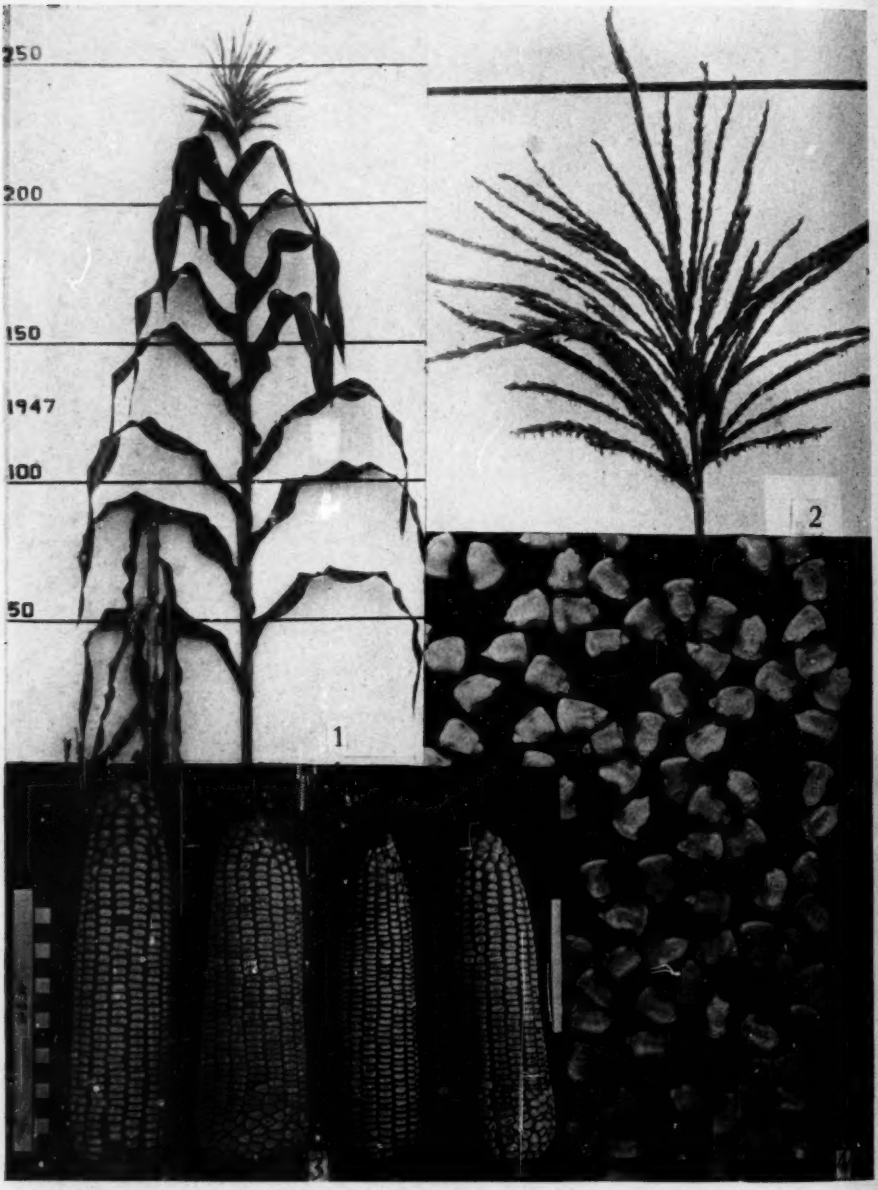
Plate 21. Shoepeg.

Plate 22. Mexican June.

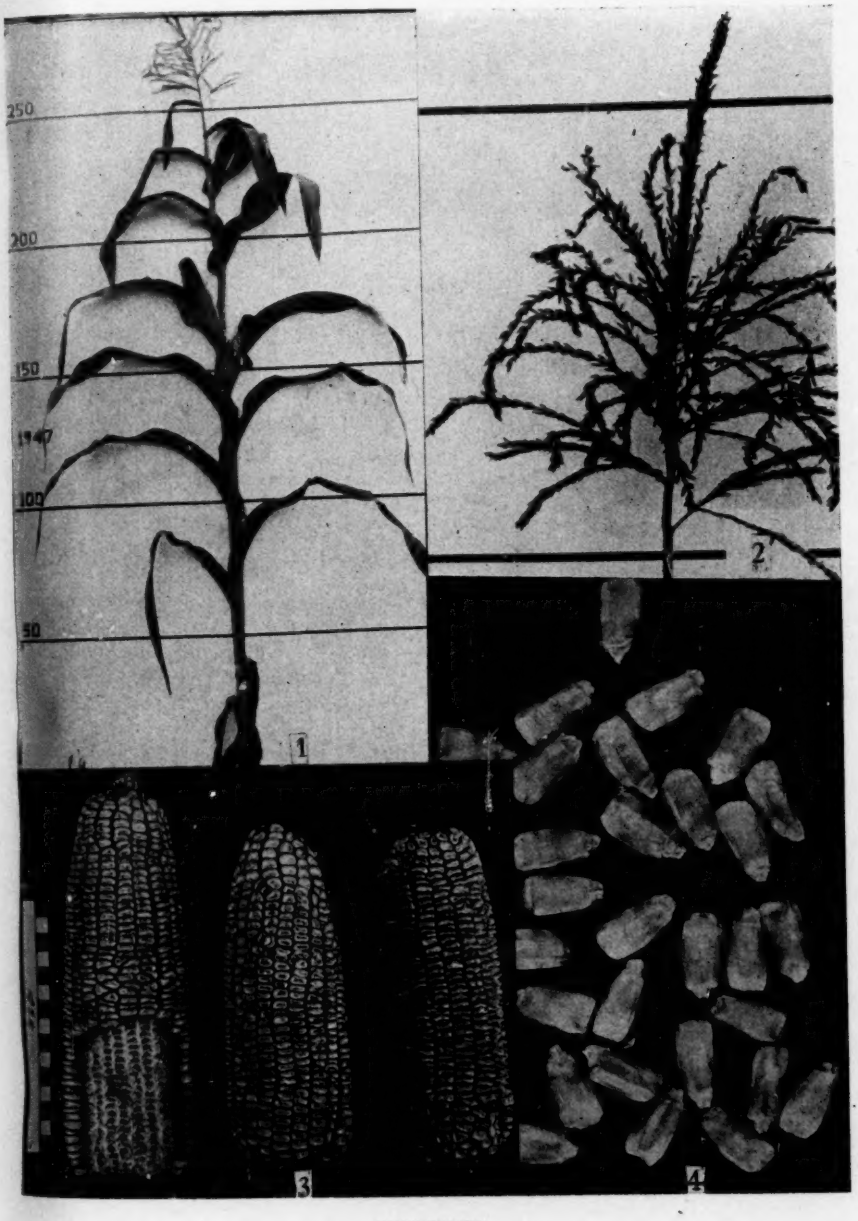
Plate 23. Hickory King.



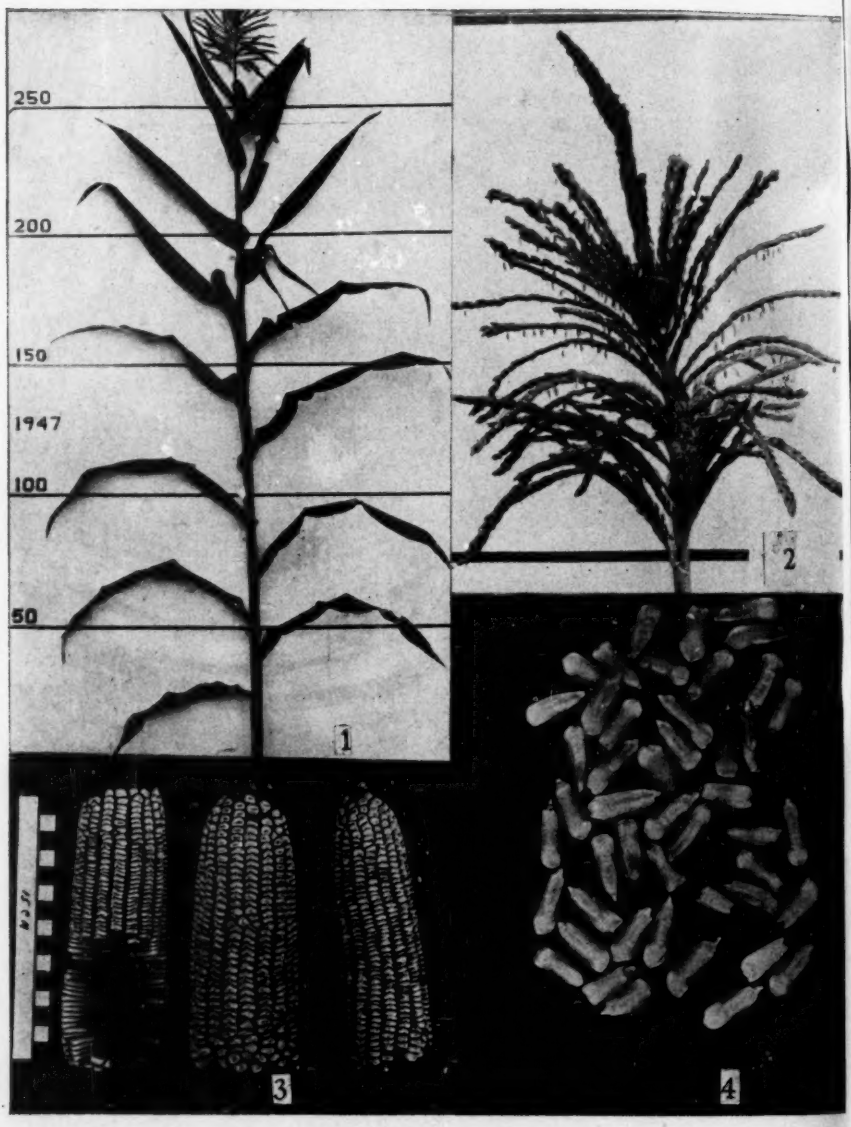
CARAWAY'S PROLIFIC



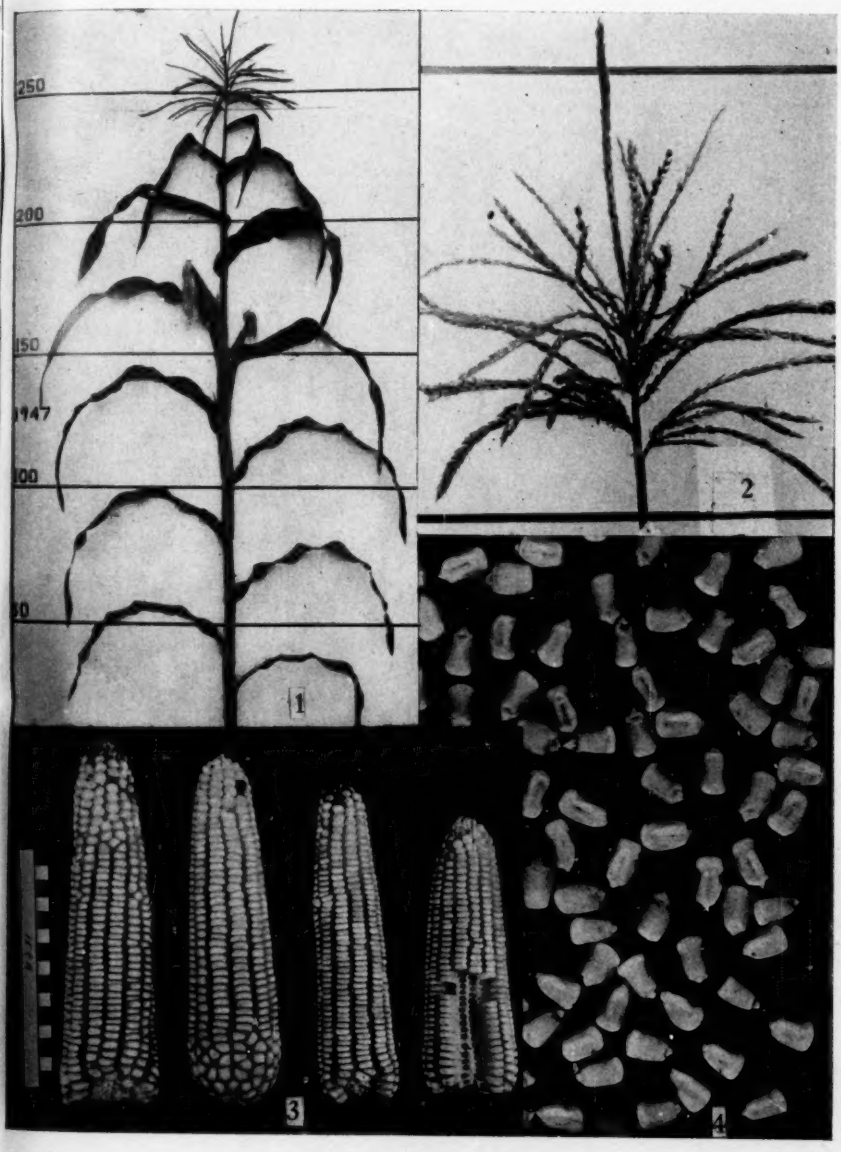
TUXPAN



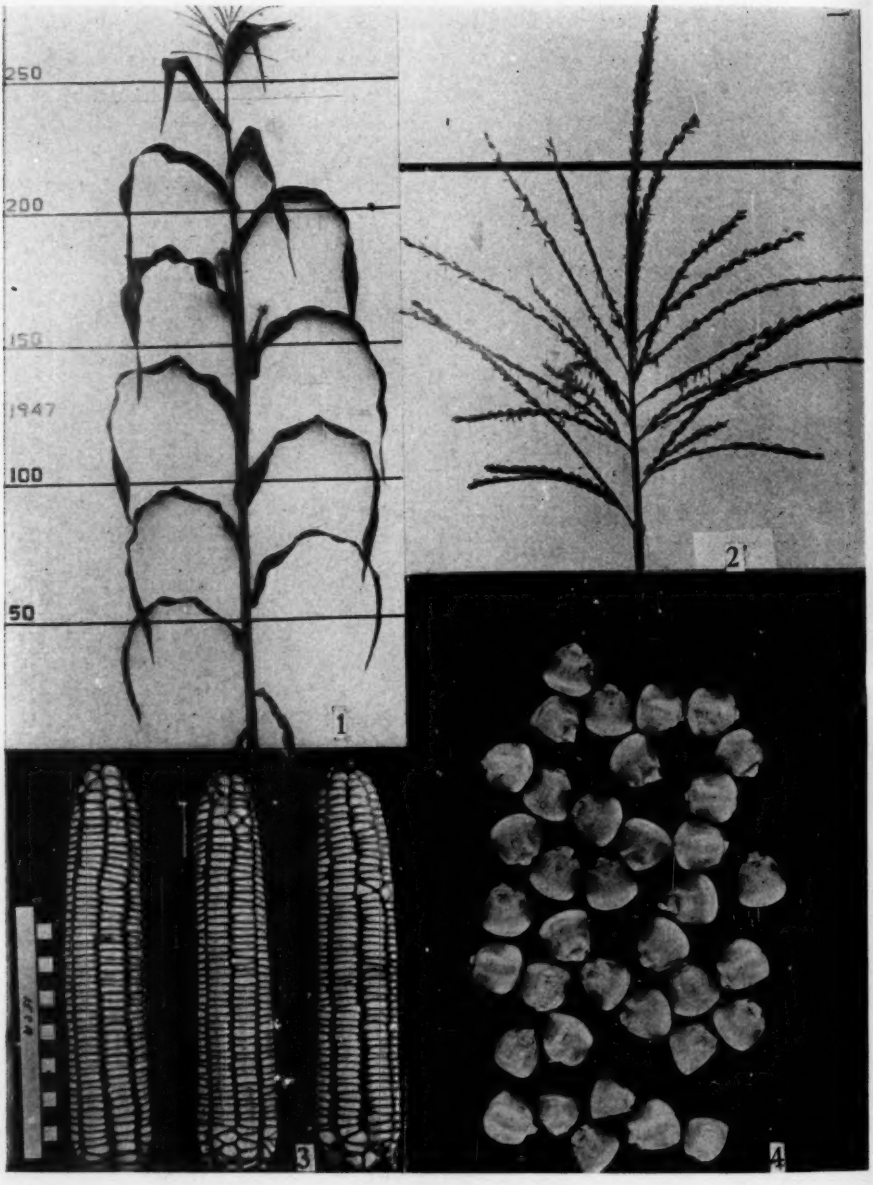
GOURDSEED



SHOEPEG



MEXICAN JUNE



HICKORY KING

THE 1947 CONFERENCE ON THE INFLORESCENCES OF *ZEA MAYS*

The inflorescences of *Zea Mays* (corn ears and corn tassels in common speech) are without parallel in the plant kingdom for the complexity of their structure and the multiformity of their variability. There is still no general agreement among botanists as to the phylogeny of these organs nor as to their morphological interpretation.

In the last decade new evidence bearing on these problems has been produced in various laboratories and by various techniques. A conference was held at the Missouri Botanical Garden on November 8, 1947, to which those known to be working on the subject were invited. The conference sessions were devoted to a series of informal discussions and demonstrations. One of the results of that conference is this series of interrelated papers. They not only present new data—they demonstrate new techniques and extensions of old ones and bring into juxtaposition facts previously considered unrelated.